Prevalence and antimicrobial resistance profile of non-typhoidal *Salmonella* in pigs in Kenya and Malawi

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Master in Philosophy

By

Catherine Nicole Wilson

February 2018

Table of Contents

Acknowledgements

There are many people to whom I am extremely grateful to for their help and support during this project. The assistance and expertise of fellow students and laboratory staff at the University of Liverpool, International Livestock Research Centre, Nairobi and the Malawi Liverpool Wellcome Trust Centre is greatly appreciated. Thanks to Professor Jay Hinton and Caisey Pulford for their assistance with the genetic analysis component of this project and James Akoko and Milicent Ojwang, 'The Flying Team', for their essential help with sample collection in Busia, Nairobi and the Chikwawa Valley.

This project would not have been possible without the funding provided by the Wellcome Trust Institutional Strategic Support Fund for this 'Research Taster Fellowship', to which I am entirely grateful.

Specific thanks to my supervisors Professor Eric Fèvre, Dr. Gina Pinchbeck, Professor Paul Wigley and Professor Melita Gordon for their help, guidance and support throughout the entire course of this project.

Abbreviations

AMOXY-CLAV	amoxicillin-clavulanate	EUCAST	European Committee on Antimicrobial Susceptibility Testing
AMP	ampicillin	GENT	gentamicin
BGA	brilliant green agar	GPS	Global positioning system
CEFOT	cefoxitin	HAC	Harlequin chromogenic agar
CTX	cefotaxime	HIV	human immunodeficiency virus
CAZ	ceftazidime	HMIC	higher middle income countries
CHLOR	chloramphenicol	ILRI	International Livestock Research Institute
CIPRO	ciprofloxacin	iNTS	invasive non-typhoidal *Salmonella*
CLSI	Clinical Laboratory Standards Institute	ISO	International Organisation for Standardisation
Dfr	dihydrofolate reductase	KANA	kanamycin
DNA	deoxyribonucleic acid	LMIC	lower middle income countries
DVS-ILRI	Department of Veterinary Services - International Livestock Research Institute	MDR	multi-drug resistant
ESBL	extended-spectrum beta-lactamase	MLN	mesenteric lymph node
EU	European Union	N	total number

NTS	non-typhoidal *Salmonella*	WGS	whole genome sequencing
PCR	polymerase chain reaction		
SGI-1	*Salmonella* pathogenicity island-1		
sSA	sub-Saharan Africa		
ST	sequence type		
STREP	streptomycin		
TET	tetracycline		
TRIM	trimethoprim-sulphamethoxazole		

Prevalence and anti-microbial resistance (AMR) profile of non-typhoidal *Salmonella* (NTS) in pigs in Kenya and Malawi

This study shows that pigs at slaughtered in Busia and Nairobi, Kenya and the Chikwawa Valley, Malawi carry a variety of non-typhoidal *Salmonella* (NTS) serovars with assorted AMR profiles, including MDR phenotypes.

Non-typhoidal *Salmonella* (NTS) has a huge global disease burden in humans, particularly in sub-Saharan Africa, and antimicrobial resistant strains are commonly found. There is suspicion that human-human transmission may not be the sole route of spread of NTS; this study investigates whether pigs may act as a potential source of transmission. The main aim of this work was to determine and compare prevalence, strains and diversity of NTS in pig samples collected at post mortem from 3 slaughter house sites; two rural (Busia, Kenya and the Chikwawa Valley, Malawi) and one urban (Nairobi, Kenya) areas. The secondary aim of this project was to determine and compare the antimicrobial resistance profile (AMR) of NTS present.

Faecal and mesenteric lymph node samples were collected from pigs at slaughter from three sites (Busia=267, Nairobi=306 and Chikwawa Valley=65). Microbiological and molecular analyses including whole genome sequencing were performed to determine the presence of non-typhoidal *Salmonella* and the AMR profile of any NTS detected. A variety of zoonotic serovars of NTS were detected in faecal and mesenteric lymph nodes of pigs at each site, including *S.* Typhimurium and *S.* Enteritidis. One isolate of *S.* Typhimurium ST313 was identified in the mesenteric lymph node of a pig slaughtered in Nairobi, Kenya and four unique isolates of *S.* Typhimurium ST19 were detected in pigs at slaughter in Nairobi. Only partial whole genome sequencing results from each of the three sites have currently been returned. Using these partial results, the current prevalence of fully confirmed NTS isolates found in pigs is 36.1% (22/61, CI 24.0-48.1%) of pigs in Nairobi (54.5% (12/22) of these from mesenteric lymph node samples and 45.5% (10/22) from faeces) and 67% (39/61, CI 51.9-76.0%) of pigs in Busia (71.8% (28/39) of these from mesenteric lymph node samples and 28.2% (11/39) from faecal samples). No whole genome sequence results have yet returned from Malawi.

Two multi-drug resistant (MDR) isolates (resistant to three or more classes of antibiotics) of NTS were detected in pigs slaughtered in Busia. 10% of current confirmed NTS isolates from Busia (4/39, CI 4-24%) and 14% of current confirmed NTS isolates from Nairobi (3/22,

CI 5-3%) were resistant to 1-2 antimicrobials. A range of different antimicrobial susceptibility phenotypes were present, including resistance to the third-generation cephalosporin ceftazidime (1/61, 1.6%, CI 0.3-8.7% of total confirmed unique NTS isolates) and the fluoroquinolone ciprofloxacin (2/61, 3.3%, CI 0.9-11.2% of total confirmed unique NTS isolates).

This study concludes that pigs in Kenya carry and shed a higher prevalence of NTS in both faeces and mesenteric lymph node than has previously been reported. Currently it appears that there is a higher level of NTS carried and shed by pigs in mesenteric lymph nodes from Busia than Nairobi. The majority of the serovars detected in this study are zoonotic, therefore have the potential to cause disease in humans and one isolate of *S.* Typhimurium ST313 was detected in the mesenteric lymph node of a pig at slaughter in Nairobi. Multidrug resistant isolates of NTS, and those resistant to 1-2 antimicrobials, have also been detected in both Kenyan locations in this study. These findings raise public health concerns as pigs in close contact with humans, both in the field and during the slaughter and meat processing process, have the potential to transmit these bacteria to humans and cause disease.

The *Enterobacteriaceae Salmonella* is a Gram-negative, rod-shaped, facultative intra-cellular bacteria. *S. enterica* and *S. bongori* are the two species which have been identified. *S. enterica* is divided into six subspecies; *S. enterica enterica*, *S. e. salamae*, *S. e. arizonae*, *S. e. diarizonae*, *S. e. houtenae*, and *S. e. indica*. Each subspecies is further subdivided into numerous serovars, distinguished by the antigens present on their outer surface membranes and classified by the Kauffman-White-Le Minor classification scheme (Grimont, 2007). Each individual serovar has different adaptations to invasion and virulence within different hosts. 'Host generalists' or 'host-adapted' species cause less virulent clinical symptoms in a broad range of animal species, often with a low mortality rate (Feasey, 2012). 'Host restricted' species of NTS have undergone genome degradation and acquisition of specific virulence genes and are therefore able to cause more invasive, systemic clinical symptoms of disease in a restricted number of species. Examples of a host-restricted *Salmonellae* are *S.* Typhi, which causes invasive disease only in humans, and *S.* Gallinarum, which causes morbidity and mortality only in chickens (Uzzau, 2000).

Non-typhoidal *Salmonellae* (NTS) are generally 'host-generalists', and this broad grouping encompasses all those serovars of NTS which cause disease, or are carried by, a broad range of hosts. This large group is extremely diverse and contains over 2500 serovars, more than 2300 of which have the ability to cause disease in humans (Porwollik, 2004).

Clinical disease and symptomatic carriage of *Salmonella* in animals

S. enterica is the main species of NTS associated with disease in warm-blooded animals. *S. bongori* has the potential to cause more frequent disease in reptilian hosts than in mammals, although rare accounts of *S. bongori* infections of humans have been reported (Nastasi, 1988, Giammanco, 2002).

Several animal species are able to carry *S. enterica* bacteria asymptomatically in their body for a period of time. In clinically affected animals, the most commonly reported clinical signs associated with disease are enteritis and systemic septicaemia. Other, rarer clinical presentations of salmonellosis in animals do occur, and include meningitis, respiratory disease, arthritis and abortion.

In animals, the majority of the more highly pathogenic serovars of NTS have a restricted host range. These include *S.* Gallinarum in poultry, *S.* Abortusovis in sheep, *S.* Cholerasuis in pigs and *S.* Dublin in cattle. Other, normally more host-generalist serotypes such as *S.*

Typhimurium and *S.* Enteritidis, are able to asymptomatically colonise the intestines or lymphoid tissue of less susceptible healthy animals, and are unlikely to cause clinical disease in these carrier animals. Therefore, prevalence of these serotypes in carrier animals is often higher than the incidence of clinical disease.

Different species of animals remain asymptomatic carriers of the bacteria for varying lengths of time. NTS may be shed intermittently in the faeces of healthy carrier pigs; a veterinary public health concern, as these animals are conduits for the spread of disease (Boyen, 2008, Wales, 2011). Sheep and cattle may carry *Salmonella* for up to ten weeks and horses for fourteen months. Pigs may shed NTS in their faeces for up to 28 weeks with no clinical signs (Wood, 1989). The rate of faecal shedding of NTS bacteria may be increased in periods of stress e.g. drought, food deprivation, crowding, parturition or surgery.

Faeco-oral transmission, either directly from other animals or from the environment, is the normal route of NTS infection. NTS invades the enterocytes and lymphoid tissue, causing intracellular infection. NTS has the ability to survive and multiply within vacuoles of macrophages (Cirillo, 1998) and so evade immune detection. In susceptible animals, when infected macrophages enter the blood stream, systemic septicaemia may occur or the bacteria may localise to other parts of the body including the meninges, reproductive tract and joints.

Salmonella infection in pigs

NTS infection in commercially raised pigs has been a concern for a number of years (Josland, 1954, O'Brien, 1966, McKinley, 1980). Pork has been found to be the second most common source of NTS for humans in food, following eggs (Pires, 2011, EFSA, 2008). A 2013 survey found that *S.* Typhimurium was the most common serovar to be isolated from pigs in the UK (AHVLA, 2014). Outbreaks of disease caused by the host-adapted *S.* Cholerasuis are now rarely reported in Europe and Australia.

It is possible for infection of pigs to occur at any point of the food chain, either on farm or during slaughter and carcass processing (Arguello, 2013). Outdoor rearing increases the likelihood of exposure to NTS bacteria through the environment, either from contaminated feed or water, soil or birds, therefore increasing the likelihood of NTS carriage or clinical signs of pigs reared in these systems. Mathole (2017) found that meat intended for human consumption in South Africa may be contaminated by NTS infected abattoir water effluents during carcass processing.

S. Typhimurium can affect pigs in one of three ways. Firstly, immunocompetent pigs can ingest *S.* Typhimurium and the bacteria pass passively through the gut without invading the host intestinal epithelium or establishing infection. Secondly, it is possible for healthy adult pigs to become actively infected and the bacteria pass through the intestinal epithelial cells and travel to the mesenteric lymph nodes within hours of infection (Berends, 1996); if the pig's immunity is good, no clinical signs of infection will be seen but the pig will carry the *S.* Typhimurium bacteria asymptomatically. Thirdly, active clinical infection can occur. Outbreaks of clinical salmonellosis in pigs on farm are rare and can normally be traced to the introduction of one infected pig to a herd. 'All-in-all-out' policies of pig stock on commercial farms helps to minimise this exposure.

S. Typhimurium and *S.* Enteritidis most commonly clinically affect piglets up to six months old. Enteritis is the most common clinical sign, with or without septicaemia. In piglets with low immunity acute illness may occur, with depression, pyrexia and death occurring within 24-48 hours. Occasionally neurological or respiratory signs may be seen. Depending upon the virulence of the infective strain, piglet mortality may reach 100%. In adults, acute enteritis alone without systemic involvement is more common. The diarrhoea is often large intestinal in nature; severe, watery and potentially containing mucus and mucus membrane tissue.

Asymptomatic carriage of NTS by pigs

The presence of NTS in the mesenteric lymph node on post mortem indicates that infection was obtained during primary production, rather than due to contamination at slaughter. *Salmonellae* are most commonly carried by pigs in the enterocytes of the large intestine and within the mesenteric lymph nodes (Nollet, 2005). This provides a source which may contaminate the abattoir environment, including other carcases, if good hygiene techniques are not employed (Morgan, 1987).

A UK wide survey of prevalence of NTS carriage in the ileo-caecal lymph nodes of pigs found that 0-29% carried the bacteria post mortem (EFSA, 2008). Studies of pig production herds in Asia, North America and Africa have indicated that carriage of NTS is common amongst pigs and normally not associated with clinical disease in these animals (Kishima, 2008, Amaechi, 2006). Should the bacteria transfer to a more susceptible host species, clinical symptoms of disease may be seen. In the field of veterinary public heath, this is likely to occur at the time of slaughter and carcass processing; contaminated pork products are thought to be a significant source of human infection (Pires, 2011, FCC, 2010). Therefore,

accurate identification of clinically healthy carrier animals is a concern for food-producing industries, as contamination of meat products with pathogenic forms of NTS may lead to outbreaks of disease.

Diagnosis in pigs

Diagnosis in cases of clinical disease in pigs includes consideration of clinical signs and isolation of the pathogen from faeces, blood or tissues, including lymphoid tissue. The most efficient method to monitor the NTS status of pigs is to carry out bacteriology of faecal samples, although serology is cheaper and easier to perform (Alban, 2012). Faecal bacteriology demonstrates current shedding and so the potential for environmental contamination or human or animal infection, therefore is an extremely important diagnostic step from a public health perspective. As faecal shedding may be intermittent, it may be necessary for more than one faecal sample to be collected and processed in order for pathogen identification to occur.

Serological tests and PCR assays are often used for surveillance and diagnosis, identifying specific serovars. Consideration should be given that a serologically positive animal may no longer be infective, as a positive serological result indicates that the animal has been previously exposed to the bacteria, mounted an antibody response, and may not be shedding the bacteria at the time of sampling (Lo Fo Wong, 2003). This could potentially lead to misclassification of the herd's current NTS status.

During post mortem of clinically affected pigs, histological lesions, including shortening of villi with a loss of the epithelium and normal intestinal architecture, haemorrhage and fibrin strands, are mostly to be found in the lower ileum, caecum and spiral colon. Histology is useful for diagnosis of an individual case to differentiate from other causes of diarrhoea, for example swine dysentery, but is an inefficient method to use in the case of herd outbreaks or discovering the source of zoonotic outbreaks of disease in humans.

Treatment of salmonellosis in pigs

Early treatment of any affected species with antibiotics is essential in cases of clinical disease in order to prevent the animal from developing a carrier status and becoming a persistent shedder of NTS following initial infection. Septicaemia should be treated with a course of oral antibiotics for at least six days, normally administered in the drinking water or intravenously. Effective antimicrobials to treat *Salmonella*-septicaemia in pigs include trimethoprim-sulphonamide, ampicillin or fluoroquinolones and third generation

cephalosporins, the latter pair of which have a broad-spectrum of action and should not be used as first-line therapy, in order minimise the number of bacteria which are affected by the antibiotics, and so minimise the number of bacteria which have the potential to develop antimicrobial resistance. Phenotypic antimicrobial resistance of NTS to ampicillin, trimethoprim, sulphonamide, tetracyclines and aminoglycosides are usually caused by antimicrobial resistance determinants transmitted between bacteria by plasmids.

Oral treatment may be ineffective due to the risk of inadequate dosing, absorption or length of antibiotic course, increasing the risk of development of a prolonged carrier state and subsequent intermittent faecal shedding of the organism, or the development of antimicrobial resistance. It is difficult to eradicate asymptomatic carrier pigs from a herd, as they become readily re-infected by environmental exposure to the bacteria, particularly when risk factors for infection such as being immuno-compromised or if concurrent disease are present.

If pigs treated with antibiotics are destined for the human food chain, the concern is that any antibiotic resistant strains of NTS may then become asymptomatically carried in the mesenteric lymph nodes or intestinal enterocytes. Selection favouring antibiotic resistant strains of NTS may occur due to treatment with an antibiotic course of inadequate duration or dosage, use of an incorrect choice of antibiotic to treat a specific infection, administration of unnecessary antibiotic treatment, or use of antibiotics at a low dose in animals to promote growth. These factors create the potential for the transfer of antibiotic resistant determinants from pigs to humans, via horizontal transfer by conjugation, transformation or transduction either in the environment or the digestive system of humans following pork consumption. This is one of the reasons that use of antibiotics as growth promotors such as tylosin or spiramycin have been banned in the EU in 2006. Although use of these antibiotics at a low dose in feed did increase the growth rate of the pigs, recurrent usage of antimicrobial drugs at a low dose within a herd has been found to hasten the development of antimicrobial resistance (Holmberg, 1984).

Control of *Salmonella* in animals

Control of *Salmonella* in pig herds can be broken up into five main categories of interventions; feed management, acidification of food or water, manipulation of the gut microbiota and vaccination (Wilhelm, 2017). Wilhelm (2017) suggested that vaccination and biosecurity management have the greatest potential to minimise NTS prevalence on an infected farm, but that only culling of infected pigs will be the strategy that eliminate

infection completely. However, *Salmonella*-free pigs are a rare commodity, and on re-stocking, the risk of re-infection is high. Generally, it has been accepted that vaccination against *S.* Typhimurium can reduce the prevalence of carriage and infection, alongside implementation of other on-farm control measures (Denagamage, 2007). The main obstacles in the control of NTS spread are the frequency of carrier animals within a herd population, the degree of environmental contamination, including carriage of NTS in the peri-domestic wildlife reservoir. Good hygiene and the maintenance of healthy herds can help to limit this, as well as preventing the introduction of infected animals into the herd. Unfortunately, *Salmonella* spp. are often resistant to removal from farms by the normal cleaning and disinfection methods (Funk, 2001). UK Government and EU supported programmes have been introduced in Europe to reduce and control infection levels in food for human consumption. In order to limit the risk of introduction of carrier pigs to a herd, it is recommended that animals should be purchased from herds with an infection-free health status and isolated for one week whilst their health status is monitored. However, there remains a risk that disease may not become apparent in carrier animals within this space of time. It should also be ensured that food for animal consumption originates from *Salmonella*-free sources.

In Denmark and other countries, it has been found that a reduction in the prevalence of *Salmonella* amongst pig herds corresponds to a reduction in the number of cases of human salmonellosis (Nielsen, 2001). A compulsory *Salmonella* spp. control programme has been implemented since 1995 throughout the country and classification of national herds was undertaken according to their NTS status. A price penalty was applied to those farms which were found to be highly infected and pigs from these farms were slaughtered separately, under increased hygiene precautions (Alban, 2002). This incentive for farmers has helped to swiftly increase the actions taken to reduce the NTS infection rate of their pigs and the result has seen the level of NTS detected in Danish pork fall from 3.5% in 1993 to 0.7% in 2000 (Nielson, 2001). There has been extremely encouraging parallel decline of the number of cases of human salmonellosis diagnosed from 1,144 in 1993 to 166 in the year 2000 (Nielson, 2001).

The prevalence of NTS in pigs in the UK is among the highest in the EU, with an in-herd prevalence of 44% (EFSA, 2009) and 21% prevalence at slaughter (EFSA, 2008). As pigs do not normally develop clinical signs of NTS infection (Kranker, 2003), the bacteria would normally only be detected during active surveillance. In Europe there is no universal

protocol for control as each pig farm differs in the location, management, facilities, host age and susceptibility. Therefore, biosecurity concerns need to be continually readdressed at an individual farm level to ensure that adequate measures are implemented (Barcelo, 1998. Amass, 2005a). To some extent vaccination of pigs against *S.* Typhimurium has been shown to help to reduce carriage (de la Cruz, 2017), however, further research needs to be carried out to develop the efficacy of this vaccination, and currently strict biosecurity measures must remain in place alongside the implementation of any vaccination programme.

Prevalence amongst pigs in sub-Saharan Africa

The prevalence of NTS in pigs has not been studied in detail in Kenya or Malawi and a coordinated national epidemiological surveillance program for NTS is currently lacking in pigs in these countries (Kariuki, 2006). Findings in Europe that the zoonotic strains of *S.* Typhimurium and *S.* Derby are the most common strains to be isolated from commercially produced pigs lead to speculation that there is high potential for zoonotic transmission of disease between pigs and humans in areas where low-intensity, free-roaming pig rearing methods are employed. To investigate this, small studies have determined the presence and antimicrobial resistance profiles of NTS in pigs in South Africa, (Mathole, 2017, Iwu, 2016), Uganda (Ikwap, 2014), Burkina Faso (Kagambèga, 2013), Ghana (Sekyere, 2015) and Kenya (Kariuki, 2006, Kikuvi, 2010) with varying results.

In South Africa, of 322 porcine faecal samples cultured, 5.9% were positive for NTS (Mathole, 2017). The prevalence of NTS amongst pigs in Burkina Faso was found to be slightly higher as NTS were detected in the faeces of 16% of the pigs surveyed (Kagambèga, 2013). In relation to antimicrobial resistance, 9.7% of *S.* Typhimurium isolates in pigs from Ashanti, Ghana, were resistant to greater than three classes of antimicrobials (Sekyere, 2015) and therefore classed as multidrug resistant.

Kikuvi (2010) investigated the prevalence of NTS isolated from 58 pigs at slaughter in the Ndumbuini abbatoir in Nairobi. These pigs were reared in the Nairobi and Kiambu regions of Kenya. 28% (16/58) of these faecal samples cultured positive for NTS. Of these 16 positive NTS isolates, 11 were detected from carcass swabs (11/16) and five from faecal samples (5/16). The detection of NTS on the outside of the carcases as well as in the faeces suggests that environmental contamination of the carcass at the abattoir during evisceration or transportation may have contaminated the samples, rather than solely direct carriage by the pigs themselves. As alluded to earlier, the presence of NTS in the

mesenteric lymph nodes of samples denotes infection of the pig during primary production rather than post slaughter during meat processing.

Ikwap (2014) looked at the prevalence of NTS in piglets in faeces in Uganda. Sampling faeces from two sites it was found that the herd prevalence of NTS was 43% and 34%, with a total herd prevalence of 39%. Following analysis, 20 serovars were identified. The predominant serovars of NTS detected were *S.* Zanzibar, *S.* Heidelberg, *S.* Infantis, *S.* Typhimurium, *S.* Stanleyville, *S.* Aberdeen and *S.* Kampala. Risk factors which appeared to increase the likelihood of infection with NTS included feeding young swine and adults together rather than feeding separately, semi-intensive (tethering and roaming) versus intensive management and washing the feeders daily rather than every other day. These could be considered as useful control points for farms to decrease the risk of *Salmonella* transmission between pigs.

As demonstrated above, there is currently a great deal of variation in the published prevalence of NTS carried or excreted by pigs by in sub-Saharan African countries. It may be that this variation truly reflects different rates of carriage by pigs in different countries. However, it is more likely that the lack of standardisation of methodologies (in regard to both field and laboratory work), sample size of the studies, inclusion of either pooled or individual samples used, and less controllable factors such as variation of seasonality during the sampling period, have influenced the final figures generated. For example, although the bacterial isolation methodologies used in Mathole and Kagambègas' studies methods were very similar, Mathole's study performed enrichment and culture from rectal and cloacal swabs, whereas Kagambèga performed enrichment of 25g of faeces in 225ml of buffered peptone water. The use of a larger volume of faeces for the enrichment stage in Kagambèga's work may have contributed to an increase in sensitivity of the laboratory work and potentially the higher prevalence of NTS detected in porcine faeces. Sekyère's study was conducted using pooled faecal samples from ten pigs, whereas prevalence of NTS for the other studies were carried out using results from individual faecal samples. Additionally, although the laboratory isolation of NTS was carried out following the ISO 6579 (2002) standard procedures by Ikwap (2014) and Mathole (2017), other authors cited above used a variety of enrichment broths, agars and tests to confirm the presence of NTS, all of which will vary in their sensitivities, affecting the accuracy of the results produced, and the reliability with which the same results for prevalence could be generated using a different method.

S. Typhiumurium and *S.* Enterica are the most widely reported zoonotic serovars of NTS which can be carried by pigs (FCC, 2010). These serovars are also those most likely to be the causal agent of invasive NTS disease in humans in Africa (Gordon, 2008, Gordon, 2001, Brent, 2006, Mabey, 1987, Kariuki, 2006, Sigauque, 2009, O'Dempsey, 1994, Lepage, 1987, Green, 1993). Therefore, the potential for zoonotic transmission from pigs to humans in sub-Saharan Africa is a concept which needs further investigation. This study aims to provide information on the prevalence of NTS in pigs as shed in faeces and detected in mesenteric lymph nodes in Kenya and Malawi, providing information about the carrier status and shedding capability of the sampled pigs.

Epidemiology and risk factors of NTS disease in humans

The contribution of different serovars of *S. enterica* to human disease varies depending on the time and geographical location of infection (Crump, 2015). NTS causes transient, self-limiting inflammatory gastroenteritis in healthy humans in higher income countries (Laupland, 2010), and is normally transmitted by the zoonotic or food-borne route. In low-middle income countries (LMICs) an invasive form of NTS is a leading cause of bloodstream infections, causing 29% cases of septicaemia across Africa and of these, 88% of the bloodstream infections diagnosed in eastern Africa (Reddy, 2010, Deen, 2012), where the case-fatality rate was 20-25% (Sigauque, 2009). The clinical symptoms in humans produced by some NTS serovars bear a closer similarity to typhoidal *Salmonellae* serovars, producing more invasive disease than others. These more invasive serovars have a differing epidemiology, clinical manifestation of disease and illicit a different immune response to infection than other, less invasive NTS serovars (Gal-Mors, 2014), particularly when patients are additionally suffering from co-morbidities, such as HIV or malaria.

In 2010, there were approximately 3.4 million cases of invasive non-typhoidal *Salmonella* (iNTS) disease worldwide, resulting in an estimated 681,316 deaths (Ao, 2015). This figure is likely to be an under-estimate (Enwere, 2006), as poor access to health-care in resource-limited countries and lack of access to diagnostic tests means that deaths and morbidity due to invasive NTS disease are under-reported (Qamar, 2015, Rutherford, 2010).

Non-invasive NTS disease is estimated to cause 93 million enteric infections and 155,000 diarrheal deaths globally each year, therefore is a major cause of diarrhoeal disease worldwide, with a proportionately much lower fatality rate than that of invasive NTS (Majowicz, 2010). The Global Enterics Multicentre Study (GEMS) did not find NTS to be one of the four major causes of moderate-severe diarrhoeal disease in Africa (Kotloff, 2013).

Invasive NTS does occur in higher income countries although at a much lower incidence than in lower-middle income areas.

In industrialised countries sources and transmission routes of NTS have already been extensively studied in humans and animals (Painter, 2013) and are better understood, particularly in healthy hosts. In lower-middle income countries, however, epidemiology studies of NTS infections are extremely limited (Morpeth, 2009) and transmission routes are understood to a far lesser degree (Feasey, 2012).

NTS is an important cause of invasive bloodstream infection in patients in sub-Saharan Africa (sSA), particularly amongst the very young (less than two years but older than six months), the elderly, immunocompromised, or those suffering from concurrent illnesses such as malaria, HIV or malnutrition (Feasey, 2010, Vugia, 2004, Varma, 2005, Crump, 2011, Parry, 2013). Elderly people may have a higher risk of developing invasive NTS due to the presence of co-morbidities which weaken the immune system, for example renal disease or diabetes (Crump, 2015). Unlike enteric fever in developed countries, NTS in sSA appears to be associated with many immunocompromising conditions (Gordon, 2008). As well as HIV, this also includes disorders of oxidative cellular killing, including chronic granulomatous disease. In patients with this chronic condition NTS has been described as the most common cause of bloodstream infections and the third leading cause of all infections (van den Berg, 2009).

Invasive NTS disease has the potential to be caused by hundreds of serovars of NTS, which have varying degrees of virulence (Jones, 2008). Patients are likely to present to hospital with a non-specific febrile illness (Gordon, 2002, Peters, 2004, Graham, 2009), therefore diagnostic tests such as blood culture are required in order for tailored treatment to be prescribed. Unfortunately, this diagnostic test can be difficult to implement, particularly in resource-limited settings (Gordon, 2011).

The NTS serovars *S.* Typhimurium and Enteritidis are the commonest serovars associated with invasive NTS disease in sSA. For 20-50% of infected children and adults suffering from invasive NTS, the clinical sign of diarrhoea may be minimal or absent (Gordon, 2002, Feasey, 2012), unlike the non-invasive disease of developed countries. Gordon (2008) found that 75% of cases of NTS bacteriaemia in Malawi were caused by *S.* Typhimurium and 21% were caused by *S.* Enteritidis, and a systematic review undertaken by Reddy (2010) demonstrated that 65% of NTS infections were due to *S.* Typhimurium and 33% due to *S.* Enteritidis.

A significant difference has been found in the age at which the different genders acquire invasive NTS, as women have been found on average to acquire the disease five years earlier than men (Feasey, 2010). HIV is a significant risk factor for the development of invasive NTS, therefore this age disparity between the sexes may also correlate with the younger age of acquisition of the HIV within the female population.

It had initially been reported that isolates of invasive NTS from Kenya and NTS causing gastroenteritis in developing countries shared similar serotype, genotype and antibiotic susceptibility patterns (Kariuki, 2006). However, Kingsley first described the novel pathovar, multidrug resistant multilocus sequence type ST313 (strain number D23580) in 2009, during the HIV pandemic, which he had discovered using multilocus sequence typing. This sequence type has a unique prophage repertoire and a degraded genome which does show some convergence with *S.* Typhi (Kingsley, 2009). Most serovars isolated from humans in Africa suffering from invasive NTS have the sequence type ST313. This sequence type has currently been found to be restricted to the African continent and appears, based on current evidence, to be host-adapted to cause invasive disease in humans (Kingsley, 2009). Work has been undertaken to determine the mechanism by which this pathovar causes invasive disease. One possible virulence gene has been found, ST313td, described in *S.* Typhimurium ST313 and also found in other NTS serovars, including *S.* Dublin (Herrero-Fresno, 2014), although the function of this virulence gene is currently unknown. Ongoing studies are continuing to elucidate further virulence genes; currently no other candidate virulence genes have been isolated from invasive NTS strains from HIV-infected patients (Preziosi, 2012). Hammarlöf (2018) has detected a single SNP difference in the gene pgtE found in a non-coding region of the *S.* Typhimurium ST313 D23580 genome, in comparison to *S.* Typhimurium ST19 4/74. This gene is part of a promotor region of the *pgtE* gene, which codes the protein PgtE, a virulence factor of *S.* Typhimurium ST313. This virulence factor is linked to the increased degradation of factor B of human complement, so contributing to serum resistance, promoting bacterial survival and dissemination. Okoro (2012) discovered that two phylogenetic lineages of *S.* Typhimurium ST313 were distinct from other *S.* Typhimurium, in that they contained genomic degradation with similarities to *S*. Typhi and the deletions and pseudogenes. These two lineages of *S.* Typhimurium ST313 have the potential to cause invasive disease in humans in sub-Saharan Africa, and both appear to carry genetic determinants for multi-drug resistance. Analysis has revealed that these two lineages developed separately, in close association with the HIV pandemic. A zoonotic source has not yet been identified, therefore it has been suggested that they have

developed to be host-adapted to humans, and transmitted, at least partly, by human-to-human transmission (Okoro, 2015).

S. Typhimurium ST19 is non-invasive and more commonly found to be the cause of diarrhoeal disease in humans in developed countries. The previously described invasive sequence type ST313 has been found to be genetically distinct from ST19, containing many pseudogenes and deletions. Therefore, it appears that two distinct clades of invasive and non-invasive *S.* Typhimurium have emerged across sub-Saharan Africa (Feasey, 2016).

Most commonly, invasive NTS occurs in children aged 2-3 years and is relatively uncommon in children between 1-4 months of age (MacLennan, 2008). The case fatality ratio among cohorts of children with invasive NTS has been found to be 20-28%, which is comparable to the case fatality ratio of other bloodstream infections (Brent, 2006, Berkley, 2005). Younger, breastfeeding children gain protection from the transfer of passive immunity in the milk or the transplacental transfer of protective IgG, therefore antibody protection is likely important for control of NTS disease in children (MacLennan, 2008, Nyirenda, 2014, Gondwe, 2010). Despite this, invasive NTS was still found to be an important cause of neonatal sepsis in Africa (Milledge, 2005), with a 62% mortality rate in neonates (0-30 days) presenting to the Queen Elizabeth Central Hospital, Blantyre, Malawi between 1996-2001. In Kilifi, Kenya, Talbert (2010) described invasive NTS accounting for 5% of blood culture isolates and 10% of cerebrospinal fluid isolates amongst 4467 children aged 0-60 days old born outside the hospital. No invasive NTS was identified from over 2000 children born in the hospital. This suggests that the domestic environment may have a role to play in the transmission in invasive NTS in this area of Kenya.

As in adults, concurrent illnesses such as malaria, HIV, and malnutrition predispose to invasive NTS in children in developing countries (Feasey, 2012). It has also been found that children who are homozygous for sickle cell disease trait are also susceptible to NTS infections (Sommet, 2013). Other inherited diseases, such as cytokine deficiencies, in particular IL-12 and IL-23, which are important to mediate intracellular killing, have been found to be associated with invasive NTS disease (MacLennan, 2004).

Overwhelming evidence has shown that cases of invasive NTS in adults are associated with concurrent HIV infection and advanced HIV disease, with 95% of NTS infected patients in some studies infected with HIV (Gordon, 2002, Watera, 2004, Profeta, 1985, Kankwatira, 2004). In children, HIV is also a risk factor for the development of invasive NTS (Berkley,

2005) and Brent (2006) found that 20% of a cohort of African children with invasive NTS were likely to be infected with HIV.

During HIV infection, Gordon (2010) found that NTS establishes an intracellular niche. Several specific immune defects were found to be associated with the development of recurrent episodes of NTS in HIV infected adults. Loss of IL-17 producing CD4 cells in the gut mucosa (Raffatellu, 2008) permits rapid invasion, dysregulating excess production of anti-LPS IgG which acts to inhibit the serum killing of extracellular NTS (MacLennan, 2010, Trebicka, 2014). The intracellular niche of NTS in HIV infected patients is likely due to the bacteria's ability to be taken into the cells before serum killing can occur (Siggins, 2014). Once NTS is within the cells it is able to survive as there is a reduction in the pro-inflammatory cytokine response in HIV infected patients (Gordon, 2010, Schreiber, 2011, Gordon, 2007). Over time this leads to recurrence of NTS disease in HIV infected individuals with mostly identical strains of NTS, which have been identified by whole genome sequencing (Gordon, 2002, Okoro, 2012). Recurrence or recrudescence of NTS disease in previously treated adults occurs in 20-30% adults with HIV, usually after a period of 4-6 months (Gordon, 2010). Infection with a new strain has only been found in 20% of recurrent cases of adults with HIV (Gordon, 2002, Okoro, 2012). Acquisition of new strains of NTS in these adults may also be postulated to occur due to human-to-human faeco-oral transmission of invasive NTS, as NTS has been found to be shed in human faeces.

The risk of NTS to patients with HIV has been found to decrease significantly in the era of anti-retroviral drug therapy (Hung, 2007), although isolates from HIV patients infected with invasive NTS have been found to be increasingly resistant to fluoroquinolones. Once effective anti-retroviral drug treatment has been established, recurrence of NTS infection appears to be less common (Hung, 2007).

In the early HIV epidemic, mortality due to concurrent NTS and HIV was found by Gilks (1990) and Gordon (2002) to be 50% in sSA. Since the improvement in recognition and management of NTS in this area, and potentially the use of more effective antimicrobial treatments and antiretroviral therapy, mortality rates appear to have fallen to 20-25% (Gordon, 2008, Arthur, 2001, Feasey, 2014).

A relationship has been identified between invasive NTS and malaria affecting both children and adults, initially in West Africa (Mabey, 1987), particularly in patients affected by severe malarial anaemia (Bronzan, 2007), severe acute malaria (Berkley, 2009) and recent malaria (Brent, 2006). Cerebral malaria has not been found to be associated with increased cases

of NTS (Bonzan, 2007). Children with invasive NTS have been found to be more likely to have received recent treatment for malaria, and have concurrent anaemia, jaundice or hypoglycaemia, which can be interpreted as complications of malaria. Mackenzie (2010) found a temporal reduction in cases of NTS amongst children when reduction of malarial cases was documented in the Gambia. A temporal relationship has also been documented by Tabu (2012), Mtove (2011) and Biggs (2014). Tabu (2012) also found a spatial relationship between malaria and NTS incidence, contrasting high- and low- incidence regions.

This association between malaria and invasive NTS disease may not be specific for NTS, as the incidence of all bacteraemias has been found to be higher in children concurrently infected by malaria (Scott, 2011).

Malnutrition is associated with invasive NTS in African children (Brent, 2006). Berkley, 2005 found this association in children, with an odds ratio of 1.68 (95% confidence interval, 1.15 to 2.44) in rural Kenya.

Invasive NTS has been found to show a strongly seasonal infection rate amongst both adults and children, coinciding with the rainy season (Gordon, 2008). This may reflect a number of factors which occur during this season: waterborne transmission of the disease, seasonal malaria transmission during periods of increased rainfall also increasing the likelihood of developing concurrent NTS, associated food scarcity and malnutrition, or a combination of these factors. Further investigations need to be conducted.

Diagnosis of NTS in humans

The gold standard for diagnosis of invasive NTS in humans is blood culture, bone marrow sample or stool culture examination, which are all techniques that require some expertise, time and diagnostic capabilities to perform and analyse the results effectively (Gordon, 2011). It is not possible to presumptively diagnose invasive NTS by clinical examination alone either in children or adults, so there is an urgent need for rapid and affordable diagnostic tools (Nadim, 2010, Graham, 2009, Gordon, 2002, Peters, 2004).

A polymerase chain reaction (PCR) assay has recently been trialled for epidemiological surveillance of invasive NTS from stool samples of children suffering from diarrhoea (Cunningham, 2010, Lin, 2010), and a multiplex PCR has been described that can reliably identify the most common typhoidal and non-typhoidal *Salmonella* causing invasive disease in West Africa (Tennant, 2010). However, as the concentration of invasive NTS in blood has

been shown to be as low as 1 cfu/ml in patients with clinical signs (Gordon, 2010), it seems less likely that PCR will be as sensitive as a rapid diagnostic test, without pre-enrichment occurring first. Further research needs to be carried out in this area.

Treatment Considerations of NTS in humans

Antimicrobial agents are not recommended for treatment of non-severe, NTS diarrhoea in healthy adults or children, but are recommended for people with evidence of sepsis or extra-intestinal infection or for specific populations at risk for bacteraemia and disseminated disease (Crump, 2015).

Invasive NTS has also been shown to establish an intracellular niche in the blood and bone marrow (Gordon, 2010). Therefore, effective treatment depends on the ability of antimicrobials to penetrate intracellular sites of infection in the macrophages of the reticuloendothelial system and gallbladder. Some antimicrobials, including gentamicin and first- and second-generation cephalosporins such as cefuroxime, appear to be effective in vitro but can be ineffective *in vivo* (Kariuki, 2015, Feasey, 2015).

For invasive NTS there is only very limited historical experience of the use of chloramphenicol compared to the fluoroquinolone, ciprofloxacin. Ciprofloxacin was introduced to the advised treatment regime following the emergence of resistance to chloramphenicol (Gordon, 2008) in treatment of invasive NTS in Africa. The reported recurrence ratio after treatment with chloramphenicol was 43% (Gordon, 2002), compared to 30% following treatment with ciprofloxacin (Gordon, 2010). This difference might be attributable to improved intracellular penetration of the fluoroquinolone. The case fatality ratio also fell gradually over the reported period, but this could be attributable to multiple effects other than the change in antimicrobial use (Gordon, 2008).

In low resource settings, commonly used antimicrobials are usually easily available to buy without prescription in pharmacies and shops and counterfeit or substandard antibiotics are also often commonly for sale (Caudron, 2008). In these countries, where the diagnosis of NTS is likely to be unconfirmed prior to therapy, only sporadic treatment may be given, which can lead to the recrudescence or development of antimicrobial resistance. The detection and monitoring of chronic carriage of NTS is vital to public health, particularly considering the widespread drug resistance of NTS.

There is little research evidence to investigate the optimal treatment regime for invasive NTS disease. It would be particularly important to investigate the effects of treatment

following initiation of anti-retroviral therapy in patients with HIV who contract with invasive NTS. Further study, ideally in the form of multicentre randomised controlled trials, would be extremely useful (Crump, 2015).

Zoonotic transmission of NTS

Currently, in sSA, small molecular studies of NTS isolated from humans and animals in the same environment have failed to find similarities between strains. Family members of sick humans have been found to carry more closely related strains (Kariuki, 2006, Kariuki, 2002) than animals or the environment. Therefore, it was hypothesized that *S.* Typhimurium ST313 had a reduced host range and infection was exclusively restricted to human hosts rather than any other species (Kariuki, 2006). However, *S.* Typhimurium ST313 has been found to have the ability to cause invasive disease in chickens (Parsons, 2013), and mice (Herrero-Fresno, 2014, Yang, 2015) offering the suggestion that zoonotic spread to some degree may be a potential route of transmission.

Asymptomatic carriage of NTS by humans occurs fairly commonly (Kariuki, 2006, Kotloff, 2013), although the contribution that asymptomatic carriage plays in the transmission of NTS, particularly ST313, between humans is currently unclear. The diversity of NTS isolated from enteric samples is likely to be wide (Paglietti, 2013) and it is likely that some of the NTS bacteria found have the potential for pathogenicity in some species.

Risk of transmission of NTS to humans in the slaughterhouse environment

Although there is currently no confirmatory evidence that *S.* Typhimurium ST313 is transmitted by animals to cause invasive disease in humans, other sequence types of *S.* Typhimurium and serovars of *Salmonella* have the potential to cause gastrointestinal disease following faeco-oral transmission. Meat destined for human consumption which is contaminated with faecal material at the slaughterhouse during carcass processing, provides an opportunity for NTS transmission to occur both to consumers ingesting the meat, and also to abattoir workers in the slaughterhouse environment, especially if the workers' hygiene standards and the availability of personal protective equipment are lacking. The meat processing industry differs between countries; in LMICs slaughter facilities vary from large industrial meat processing plants to unregulated facilities in rural areas (Clottey, 1985). Often, in areas where the process is unregulated, there will also be a lack of suitable or affordable materials for the processing and transportation of meat (Mann, 1984). These factors, in combination with the lack of education and understanding of the risk of transmission of food bourne disease, means that hygiene conditions in rural

slaughterhouses in LMICs are often poor (Mann, 1984, FAO, 2010), offering a greater opportunity for faeco-oral transmission to occur.

Hazard Analysis and Critical Control Point (HACCP) is the name for a systematic, preventative approach to food safety developed in the 1950s by the Pillsbury Company, USA. In HMICs, HACCP programs are regularly in place at all stages of the food processing chain and these stages are regulated by official bodies. In Kenya and Malawi, particularly in the rural communities, there are numerous failings present during the slaughter process, which would ideally be more strictly regulated as part of the HACCP programme. These include inadequate infrastructure, poor hygiene, lack of ante and post mortem inspection and inadequate training of slaughterhouse workers (FAO, 2010, Herenda, 1994). As well as increasing the risk of contamination of the meat with microbes prior to entry into the food chain; this lack of infrastructure at the level of the slaughterhouse also endangers the health of any slaughterhouse workers or others handling the raw carcasses due to the increased potential for transmission of zoonotic food borne disease.

There are seven main principles of HACCP. The overall aims are to identify risks and hazards in the food processing pathway as well as critical control points where these hazards can potentially be controlled; establish critical limits for each of the critical control points, such as bacterial load of meat or water temperature; and creation of a monitoring plan, followed by detailing corrective actions that should be taken should the levels of critical control points deviate, followed by establishing a system for ongoing monitoring and record keeping.

It is expected that in the more urban slaughterhouses in Nairobi, with a higher throughput of animals with meat supplied to outlets all over Kenya, that the HACCP steps will be more firmly regimented, records will be kept of post and ante-mortem inspection and training offered to the staff to facilitate the hygienic handling of meat. In the rural slaughterhouses of Kenya and Malawi, it is anticipated that the steps to monitor the critical control points will not be in place. In fact, Cook (2017) found that protective clothing was only worn by 55% of workers in slaughterhouses and only 35% of the buildings had hand washing facilities in place. The purpose of protective clothing is primarily to protect the meat product from contamination, and also to protect handlers of the meat from zoonoses that can be transmitted directly, such as leptospirosis and brucellosis. In all rural slaughterhouses surveyed in Western Kenya, Cook (2017) found that slaughtering, bleeding, skinning and evisceration were performed in the same area of the slaughterhouse, a

procedure known as 'batch slaughtering'. Ideally, a HACCP control point would represent a division in the slaughterhouse between the dirty' (killing, bleeding) and 'clean' (evisceration and splitting) operations to prevent contamination of the carcass. (Codex-Alimentarius-Commission, 2005). Cook (2017) also found that the majority of carcass processing took place on the ground, a HACCP which would be controlled by regulation in areas where stricter control is placed over the steps of the slaughtering process. It is stated by international guidelines that hot and cold water should be available for cleaning the equipment and workers' hands, along with soap (Codex-Alimentarius-Commission, 2005). Considering this as a HACCP, it is not expected that this facility will be available in the more rural areas. Hand washing plays a role in protecting the slaughterhouse workers against the direct transmission of bacterial pathogens, as well as decreasing the risk of meat contamination.

In Kenya, the meat industry is regulated by the directorate of Veterinary Services under the State Department of Livestock in the Ministry of Agriculture, Livestock and Fisheries (Gov. of Kenya, 2012). In 2012, a revised Meat Control Act was put in place was introduced to standardise the meat industry across the country. The components of this revised Act covers building layout, personal hygiene, carcass handling, waste management and meat inspection. The Meat and Meat Products Act of Malawi was released in 1975 provides standards for the Meat Industry in the country to follow. This Act has not yet been revised since this time.

Consumers, as well as slaughterhouse workers, are also at risk from meat which has been faeco-orally contaminated during the slaughtering and carcass-dressing process. In more rural areas of Kenya and Malawi, pork is often cooked slowly over a flame with no temperature regulation. The meat is normally heated for a long time until it is bought by a consumer, and the variability in the length of time of cooking can determine whether or not any microbes contaminating the surface of the meat from the slaughterhouse are killed. Should the temperature be low, or the meat cooked for an inadequate length of time, microbes may survive and act as a source of food borne zoonotic infection.

Antibiotic resistance mechanisms of NTS

It has been found that recent use of antimicrobials may facilitate NTS infection in humans (Delarocque-Astagneau, 2000). There has been widespread use of antimicrobials to halt disease caused by NTS, which has led to the development of antimicrobial resistance displayed by this bacteria worldwide (Mathole, 2017).

Resistance to the commonly used antimicrobials such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole are commonly detected on plasmids (Butaye, 2006). In a study by Makoka (2012), 77.4% of NTS isolates from humans were resistant to ampicillin, 81.5% to trimethoprim-sulfamethoxazole and 71% to chloramphenicol. Ampicillin resistance of NTS is often mediated by ß-lactamases (bla_{PSE} and bla_{TEM} genes). Several mechanisms can be associated with chloramphenicol resistance; there are three types of chloramphenicol acetyltransferases described in Gram-negative bacteria (CATs, Type I-III). The most frequent to be determined in *Enterobactericeae* is CAT I (Arcangioli, 2000). As folate pathway inhibitors, trimethoprim and sulfamethoxazole are affected by resistance genes acquired by bacteria, which have a role in the folate pathway but do not bind these compounds. The *dfr* (dihydrofolate reductase) genes of *S.* Enterica encode trimethoprim resistance and the *sul* genes (*sul1* or *sul2*) encode sulfamethoxazole resistance (Glenn, 2011).

The emergence of *Salmonella* spp. phenotypes resistant to the quinolones, e.g. ciprofloxacin and nalidixic acid, and the cephalosporins, e.g. ceftriaxone, has raised public health concerns worldwide, including in Blantyre, Malawi, where extended spectrum beta-lactamase (ESBL) and fluoroquinolone-resistant NTS isolates have been previously identified (Gray, 2006). Cephalosporins are important for treatment of invasive NTS, particularly in children, in which age group the use of fluoroquinolones should be avoided due to the occurrence of adverse side effects. ß-lactamases of the ESBL or AmpC type were first recorded in the mid-1980s, conferring resistance to the extended spectrum cephalosporins, and some of the earliest reports were from North Africa (Miriagou, 2004, Ben Hassen, 1990). Cephalosporin antibiotics act by disrupting bacterial cell wall synthesis by disrupting the penicillin binding proteins and the cross-linking of peptidoglycan (Marshall, 1999). ß-lactamases commonly mediate resistance, including to the extended-spectrum cephalosporins e.g. These cleave the beta-lactam ring so inactivating the antibiotic. There are three groups of ß-lactamases which mediate resistance to the extended-spectrum cephalosporins: extended spectrum beta-lactamases (ESBLs), carbapenemases, and AmpC-type- ß-lactamases (Paterson, 2005, Philippon, 2002). In *Salmonella* spp., the predominant cause of cephalosporin resistance is due to the AmpC plasmid-mediated ß-lactamases, cephamycinases (CMY) which are encoded by the bla_{CMY} genes. Resistance due to ESBLs has occurred more often in non-typhoidal than typhoidal *Salmonella* strains (Crump, 2015). Resistance due to ß-lactamase enzymes may spread horizontally as the genes involved are commonly encoded on mobile genetic elements which include plasmids, transposons and

integrons. Enzymes of CMY and CTX-M types ($bla_{CTX\text{-}M63,}$ $bla_{CTX\text{-}M14,}$ $bla_{CMY\text{-}2}$) have been noted in *Salmonella* in Thailand (Pornruandwong, 2011, Sirichote, 2010) and some of these isolates also demonstrate resistance to fluoroquinolones in humans (Sirichote, 2010, Sirichote, 2010, Kulwichit, 2007), which is particularly worrisome as more expensive or difficult to obtain drugs may be required for treatment of these cases, such as the carbapenems or tigecycline.

Fluoroquinolone antimicrobials inhibit bacterial replication by affecting the enzymes DNA gyrase and topoisomerase IV. The subunits of these enzymes are encoded by *gyrA*, *gyrB* and *parC* and *parE* genes (Hopkins, 2005). Reduced susceptibility or resistance of NTS bacteria to fluoroquinolones may be induced, dependent upon the number of mutations acquired in the regions of these genes (Hopkins, 2005, Turner, 2006). Plasmid mediated mechanisms have also been described to demonstrate low levels of resistance to fluoroquinolones e.g. *qnrA* which has been detected in NTS (Strahilevitz, 2009, Martinez-Martinez, 1998).

Genes which encode proteins which effect non-enzymatic mechanisms of resistance, e.g. *cmlA* and *floR*, have also been detected in *S.* Enterica (Arcangioli, 2000, Glenn, 2011).

Recently, isolates with extensively multi-drug resistant phenotypes have emerged in humans. *S.* Typhimurium isolates resistant to 12-15 antimicrobial agents, from 6-7 drug classes including the cephems, have been reported in Malaysia and Vietnam (Tiong, 2010, Vo, 2010, Benacer, 2010) and isolates resistant to 6-7 antimicrobial agents, compromising of 3-5 drug classes, have been detected in Thailand (Wannaprasat, 2011).

A multi-drug resistant phenotype of *S.* Typhimurium was first detected in UK in the 1980s, closely associated with the specific phage-type DT104 (Threlfall, 2002). This phenotype displays resistance to five antimicrobial agents; ampicillin, chloramphenicol, streptomycin, tetracycline and sulphonamides. This resistance phenotype was referred to as ACSSut. Since this time, although initially ACSSut was found in several other European countries, the phenotype prevalence has since declined (Parry, 2003, Threllfall, 2000). The resistance genes for this phenotype are often clustered together on a chromosomal genetic element *Salmonella* Genomic Island 1 (SGI-1). Initially this element was detected in *S.* Typhimurium DT104, but has since been noted in a variety of serovars (Levings, 2005, Doublet, 2005, Hall, 2010). To be more exact, SGI-1 is classically a 14kb sequence bracketed by two integron structures containing the following antimicrobial resistance genes: bla_{PSE}-1 conferring

ampicillin resistance, *floR*; chloramphenicol and florfenicol, *aadA2* to streptomycin, *sul1* to sulphonamides and *tetG* to tetracycline (Boyd, 2002).

NTS isolates resistant to the carbapenems have been reported in China, Columbia, Pakistan and the US and variants displaying resistance to cephalosporins and carbapenems (KPC-dependent resistance) have been detected since 2009. The enzyme NDM-1 carbapenemase and New Delhi metallo-beta-lactamase-1 (NDM-1) enzyme were the most common in these isolates (Jure, 2014, Rodriguez, 2014, Irfan, 2015, Rasheed, 2013, Huang, 2013); concerningly, these isolates were also found to be resistant to most aminoglycosides, trimethoprim-sulfamethoxazole and azithromycin (Le Hallo, 2013).

One study of a commercial pig herd and one of smallholder pig farms in South Africa has been currently undertaken to investigate antimicrobial resistance of NTS carried by these animals in sub-Saharan Africa (Iwu, 2016). Mathole's more rural study (2017) found that of 322 free-range healthy pigs, 19 isolates of NTS were detected, of a diverse range of serotypes. All NTS isolates were sensitive to cefotaxime, enrofloxacin, florphenicol and polymixin B. Most of the NTS isolates (66.7%) were resistant to at least one antimicrobial, which was predominantly trimethoprim, followed by ampicillin. No multi-drug resistant isolates (resistant to three of more classes of antibiotics) were detected.

Of the commercial pig herd in Iwu's study (2016), 258 presumptive NTS isolates were detected from faecal samples from 500 adult pigs, a much higher prevalence than Mathole's study (2017). Reasons for this higher prevalence could be related to closer contact of the animals in more intensive livestock rearing systems than the less commercial small-holding units. Forty-eight of these isolates underwent antimicrobial susceptibility testing using the disc diffusion method. Using PCR with primer sequences for the bla_{ampC}, *tetA* and *strA* genes, the authors found that approximately 54% of the isolates were positive for the bla_{ampC} antimicrobial resistance gene (further details of this gene were not identified), 61% for the *tetA* gene and 44% for the *strA* gene. 16% of the isolates were resistant to 11/18 antibiotics tested, which included third generation cephalosporins and fluoroquinolones. All isolates were resistant to tetracycline, 75% to ampicillin and 75% to streptomycin. Four of the resistant isolates demonstrated the previously mentioned gene-encoded resistance pattern of DT104, ACSSut (ampicillin, chloramphenicol, streptomycin, sulphonamides and tetracycline). This multidrug resistance profile has been isolated from pigs and pig products in several countries (Kishima, 2008) and is one of two most common MDR patterns to be detected in both pigs and humans (Gebreyes, 2009), and therefore

poses a risk to human and animal health. These results are more similar to results observed in commercial pig farms in other parts of the world, such as New Zealand (Gebreyes, 2000) and Spain (Agustin, 2005). Antimicrobials are frequently used as growth promoters and in the treatment of infections in South Africa. The results of this study show that antimicrobial resistance is a concern in countries in sub-Saharan Africa. Although commercial pig production may confer a greater risk of multi-drug resistance, likely due to overuse of antimicrobials as growth promotors and potentially prophylactically to treat disease, further investigation needs to be undertaken on this continent.

Preventing NTS disease in humans and pigs

It is hoped that in the future a safe, efficacious and reasonable priced vaccine will be developed to prevent invasive NTS infection of both pigs and humans. *Salmonellae* are facultative intracellular bacteria, therefore a live attenuated vaccine, which stimulates a cell-mediated immune response is necessary to offer protection against clinical disease and intestinal colonisation in both species. However, the common occurrence of invasive NTS disease in already immunocompromised human patients presents a challenge for the development of an effective vaccine.

Currently, efforts are underway to evaluate the development of a few candidate vaccines for humans and any introduction of a vaccine is likely to be some time in the future (MacLennan, 2014, Martin, 2014). The role of antibody in serum and intracellular oxidative killing of invasive NTS in African children has been postulated as a potential important target for vaccine development (MacLennan, 2008, Tennant, 2010). There are currently vaccines available to counter NTS in poultry and livestock, but no NTS vaccines are available for humans.

Pigs may be vaccinated against NTS to fulfil at least one of two aims; to gain protection from clinical disease or to reduce the degree of subclinical shedding of NTS bacteria. Control of the specific serovars causing clinical disease may require the use of a killed vaccine prepared to provide protection to naïve pigs during a specific outbreak, in addition to other more biosecurity-focussed control measures including movement restriction. Vaccination to reduce subclinical shedding of NTS by pigs in faeces in the context of continuous herd reinfection requires vaccination against a number of serovars, which may originate from different antigenic groups. *S.* Typhimurium shows a different pattern of invasion, multiplication and spread than *S.* Cholerasuis (Boyen, 2008) and protection from subclinical shedding of both serovars would involve two different antibody stimulants to be

present in the vaccination. Routine herd monitoring for NTS is often carried out by serological testing in Europe, and the differing serological responses of vaccinated to unvaccinated pigs should be considered when using this test to monitor a herd's NTS status, as positive serological results in vaccinated animals may confuse the true prevalence of NTS carriage within a herd.

In developing countries, reduction in the frequency of co-morbidities affecting the human population such as HIV, malaria or other immune-suppressive diseases will also be necessary in order for the incidence of clinical NTS disease in humans to reduce. Policy management to address priorities in increasing food safety and hygiene in animal production and meat processing in LMICs should be addressed. Currently, it can be found that these policies are only loosely implemented in some slaughterhouses in LMICs, as the infrastructure for regulation that can be put in place in higher-middle income countries (HMICs) is not possible in all LMIC settings.

In industrialised countries, regulation of food-safety from 'farm-to-fork' is fundamental to the control of NTS as transmission is most commonly zoonotic or environmental. Steps to reduce the frequency of the transmission within these countries are common, and in the EU, enforced by EU-wide directives. These steps include rodent control, effective biosecurity implementation, involving both access to the farm and whilst on farm, cleanliness of vehicles and personnel, appropriate waste management strategies, feed based interventions, which involve food provided to be of low-pH, so discouraging growth of NTS within the gastrointestinal tract, vaccination against NTS, a good hygiene policy on farm and limited movement and mixing of the herd with unknown animals (Andres, 2015).

Aims

.

The primary aim of this thesis was to determine and compare prevalence, diversity and strains of NTS in pig samples collected at post mortem from 3 slaughter house sites; two rural (Busia, Kenya and the Chikwawa Valley, Malawi) and one urban (Nairobi, Kenya) areas.

The secondary aim was to determine and compare antimicrobial resistance profiles of NTS present.

Chapter 2 describes the design of the study and the sampling protocol and the microbiological and molecular methods employed to determine the presence of NTS prior to whole genome sequencing.

Chapter 3 presents results of prevalence, diversity and strains of NTS and the antimicrobial resistance phenotypes detected.

The concluding discussion discusses the findings of this thesis in the context of current published research.

Chapter 2: Materials and Methods

2.1 Study overview

Faecal and mesenteric lymph node samples were collected from pigs at slaughter at three study sites in sub-Saharan Africa; Busia, in rural Kenya; Nairobi, in urban Kenya and the rural Chikwawa District; Malawi. This cross-sectional study was carried out over an eight-week period in Kenya (October-December 2016) and a two week period in Malawi (April-May 2017). NTS were isolated from the samples and the isolates' susceptibility to twelve antimicrobial agents was determined. All NTS isolates were submitted for whole genome sequencing and presence of antimicrobial resistance determinants investigated.

Ethical Consent

Ethical consent for this study was obtained from the University of Liverpool Veterinary Research Ethics Committee (Reference number VREC465), the International Livestock Research Institute Ethics Committee, Nairobi, Kenya (IACUC reference number 2016.19) and the College of Medicine Research Ethics Committee, Malawi (Reference number P.02/17/2124).

2.2 Study Population

Pigs selected were those presenting for slaughter on the day of sample collection at designated slaughterhouses in the three study sites. Designated slaughterhouses in each location were chosen as those which were geographically convenient for the study team to reach daily. In Busia and Malawi, all sampling locations included in the study were within a ninety-minute drive from the laboratory where processing was to take place, which enabled samples to be collected from all slaughter slabs daily. All pigs presenting to the slaughterhouse on the day of sampling were eligible to be included in the study. Informed consent was obtained from all owners, Traders and the slaughterhouse owners. Pigs were sampling according to the capacity of the fieldworkers. In the quieter slaughterhouses, all pigs were samples, but in the Ndumbuini slaughterhouse, Nairobi, where throughput is high, as many pigs as possible were sampled daily, but some pigs were left unsampled if at the time of carcass dressing the fieldworker was busy logging a previous sample. This occurred randomly and there was no selection carried out by the fieldworkers as to which pig was missed or sampled.

In Nairobi, Ndumbuini slaughterhouse was sampled, which is located in the Kikuyu District of Kiambu County on the peri-urban fringe of Kenya's capital city (Map V, Appendix). This is a dedicated swine slaughterhouse receiving pigs from the whole country and is the main supplier of most of the butcheries in central Nairobi. No processing of pork, other than cutting, is carried out on site. The slaughterhouse has a capacity of 40-50 pigs slaughtered per day. The majority of these pigs originate from Kiambu and surrounding areas; although some originate from further afield in Kenya (see Table I, Appendix). Often the pigs delivered to this slaughterhouse have been reared in a more commercial, intensive environment than pigs in Busia or Chikwawa. Slaughtering takes place via the 'sticking method' following electrical stunning across the pigs' temples. A commercial, intensive system, with approximately 20 assistants caters for the processing and dressing of 40-50 pigs daily.

In Busia samples were collected from seven different slaughter slabs, located within an hours' drive of Busia town. These slaughter slabs were Bumala, Matayos, Mundika, Mungatsi, Nambale, Tangakona and Funyula (see Map I, Appendix). Each slaughter slab does not run operations on a daily basis, generally functioning on specific market days of the local town, therefore visits to the individual slaughter slabs were made on specific, functioning days. The slaughter slabs cater for 1-15 pigs each per working day, normally delivered to the slaughterhouse via individual Traders on motorcycles who have collected the animal from the original village in which it was reared. Pigs in the Busia area are reared via low-intensity methods as free-roaming animals, scavenging around the homesteads and villages to gain access to food (Thomas, 2013). Slaughtering takes place using the sticking method. No stunning was employed in the Busia region.

In the Chikwawa district, Malawi, samples were collected from pork butcheries, to which slaughtermen brought the meat samples. For cultural reasons, slaughtering of pigs in the rural Chikwawa district occurs in the compounds of the owners. One compound was visited in order to obtain faecal and mesenteric lymph node samples from three pigs, however logistics dictated that following instruction from the Field Team, the majority of samples were collected by slaughter men at the point of slaughter prior to carcass dressing, and brought to the butchery within two hours of slaughter, where they were collected and transported at 4°C to the laboratory for processing. Sample collection took place from butcheries located in three towns in the Chikwawa District; Chikwawa town, Nchalo and Ngabu (see Map III, Appendix). A variety of slaughtering methods was employed to slaughter the pigs in the Chikwawa District, these include sticking and hanging. No stunning techniques prior to slaughter were employed.

2.3 Sample size calculation

Sample size was calculated using an estimated prevalence of carriage of non-typhoidal *Salmonella* in the pig population of 10% (Kariuki, 2002) using a 3% precision and 95% confidence interval. Adjustment for clustering at the three sample sites was made using the formula $n^1 = n(1+p(m-1))$, see below. The intra-class coefficient was estimated to be 0.1 and samples were taken from three sites.

Formula $n^1 = n(1+p(m-1))$

New sample size = 384(1+0.1 (3-1) = 461 samples.

Therefore 461/3=154 pigs to be sampled from each of the three sites.

n= sample size using estimated prevalence of 10%, 95% CI and 3% precision and a large population size

p= ICC

m= number of sites where samples will be taken

n^1 = sample size corrected for clustering

2.4 Sample collection

Faecal and mesenteric lymph node samples were taken from pigs *post mortem*. From each pig sampled, between 1-25g faeces was taken manually from the rectum. Once the entire gastrointestinal tract had been removed during meat processing between 1-4g of mesenteric lymph node tissue was excised using a sterile scalpel blade. This tissue sampled was composed of at least five individual mesenteric lymph nodes located in various different areas of the mesentery of the small and large intestine. Approximately three lymph node samples were taken from the mesentery of the ileum and jejunum and two samples were taken from the colonic mesentery, to total 1-4g.

The samples were placed into individual sterile containers which were collected in a chilled box (4°C). Samples reached the laboratory and were processed within four hours of collection.

Additional data were collected on paper and electronically using a 'Field Information Support Tool' developed from a Case Report Form by the Kestrel Technology Group on a

Nexus 5 Android device. This questionnaire included name of the village where the pig was reared, previous antibiotic treatment if known, age, sex and breed of pig as well as method of transport of the pig to slaughterhouse, where possible, from either the owner of the pig or the tradesman who had brought the animal to the slaughterhouse. The GPS location of each of the slaughterhouses was recorded using a Nexus 5 device.

2.5 *Salmonella* Isolation

Samples were processed once they reached the laboratory within four hours of the initial sampling. NTS isolation and culture (based on Standard Procedures, International Organisation for Standardisation (ISO 6579:2002, Ikwap, 2014), serotyping and antimicrobial susceptibility testing took place at the Directorate of Veterinary Services-International Livestock Research Institute, (DVS-ILRI) Zoonoses Laboratory, Busia, ILRI Laboratories, Nairobi and the University of Liverpool-Wellcome Trust laboratories, based at the Malawi-Liverpool Wellcome Trust Centre, Blantyre, Malawi. ISO is a global federation of national standards bodies. Publication of an International Standard requires approval by at least 75% of the member bodies.

The exterior of the surface of each of the mesenteric lymph node samples was placed briefly into a flame to remove any residual exterior contamination. 1g of mesenteric lymph node (composed of parts of five mesenteric lymph nodes from multiple locations in the gastrointestinal tract) and 1g of faeces from each pig were placed into separate stomacher bags containing 9ml 2% buffered peptone water. Samples were homogenized manually and placed into sterile bijou bottles. Each sample was incubated for 18 hours at 37°C. All of the media used to undertake the protocol was purchased from Oxoid Ltd., Basingstoke, UK, apart from the Harlequin ABC Salmonella agar which is manufactured by Lab M, Bury, UK.

Following pre-enrichment, 0.1ml of each sample was placed into a sterile bijou containing 9.9ml Rappaport-Vassiliadis broth. These were incubated for 24 hours at 42°C.

Each enriched sample was inoculated onto both Brilliant Green agar and Harlequin ABC *Salmonella* plates. All plates were incubated for 24 hours at 37°C.

The plated growths were assessed and those colonies with the appearance of *Salmonella* growth were isolated. On the Brilliant Green agar plates this included pink colonies and green colonies from the Harlequin ABC *Salmonella* agar plates. These positive colonies were inoculated onto nutrient agar plates and incubated for a further 24 hours at 37°C prior to antisera agglutination and antimicrobial susceptibility testing.

2.6 *Salmonella* confirmation by serotyping

A small part of the suspect NTS colony was taken from the overnight culture and mixed thoroughly with a drop of sterile saline on a clean glass slide using a sterile microloop. One drop of *Salmonella* antisera (Poly O antigen and H Phase 1 and 2 or H Phase 2 antigens, Prolab, Ontario, Canada) was added to one aliquot of bacterial suspension and further mixing with a sterile loop occurred. The slide was gently tilted back and forth for one minute and agglutination was assessed for under normal lighting conditions. Where agglutination was seen to occur the isolate, taken from the nutrient agar culture, was stored into a microbank tube, frozen at -80°C and transported to the UK, where PCR was carried out followed by whole genome sequencing for those samples that yielded a positive PCR result. Antisera testing was carried out for all isolates using the O antigen, and all isolates from Busia using both the O and H antigens.

2.7 Antimicrobial Susceptibility Testing

The disc diffusion method was carried out in accordance with the European Committee on Antimicrobial Susceptibility Testing guidelines (EUCAST, 2016). All assumed isolates of NTS, positive on agglutination testing, underwent antimicrobial susceptibility testing. Using an inoculating loop, 3-5 isolated colonies were taken from the plate. The colonies were added to 1ml sterile saline to create a solution of 0.5 McFarland standard (McFarland, 1907). The solution was mixed well and a sterile cotton-tipped swab was placed into the suspension. The entire surface of two Mueller Hinton plates was inoculated by streaking the solution over the entire area. Antimicrobial discs were applied within 15 minutes of inoculating the Mueller Hinton plate. Twelve antimicrobial discs were placed firmly onto the agar. The antimicrobial discs and their potencies were: ampicillin (10ug), amoxycillin-clavulanate (20ug/10ug), streptomycin (10ug), ceftazidime (30ug), kanamycin (30ug), ciprofloxacin (5ug), cefoxitin (30ug), trimethoprim-sulphamethoxazole (25ug), cefotaxime (30ug), gentamicin (10ug), chloramphenicol (30ug) and tetracycline (30ug) (Behl, 2017, Choudhard, 2013, Kebede, 2016, Maka, 2014 Poudel, 2014). Kanamycin was not available to use as part of the disc diffusion protocol in Busia, therefore gentamicin was used in its stead during antimicrobial susceptibility testing of NTS from this location. Plates were incubated at 37°C for 16-18 hours. After this time, the diameter of the zones of inhibition was recorded in millimetres and recorded as resistant, intermediate or sensitive in accordance with the EUCAST guidelines (EUCAST, 2016).

2.8 Polymerase Chain Reaction

All isolates from Busia and Nairobi slaughter slabs which gave positive results on the *Salmonella* spp. antisera test were submitted for PCR to confirm the presence of NTS. This was carried out in the University of Liverpool, Leahurst Campus Laboratories, United Kingdom. PCR testing was not carried out on isolates from Malawi due to delays in shipping of samples to the UK.

Preparation of DNA

A loop of culture was taken from the microbank tube, inoculated onto nutrient agar and cultured for 24 hours at 37°C. Using a sterile microloop, 3-5 colonies of this overnight culture were placed into a 1.5ml microcentrifuge tube containing 300ul of ultra-pure water. The samples were boiled at 100°C using a heat block for 10 minutes in order to lyse the cells and release the DNA.

After the solutions had cooled, the NanoDrop machine was used to demonstrate that sufficient DNA was present prior to the PCR testing.

Primers

The primers to detect presence of the tetrathionate reductase gene which is constitutively present in all *Salmonella* spp. (Malorny, 2004). The primers used were ttr-4 and ttr-6, made up and supplied by Eurofins (www.Eurofinsgenomics.eu).

Designation	Sequence	Melting Temperature (°C)
ttr-6 (forwards)	CTCACCAGGAGATTACAACATGG	57
ttr-4 (backwards)	AGCTCAGACCAAAAGTGACCATC	58

Table 1. DNA sequence of primers used

PCR Assay

The PCR reaction mixture contained My Taq^{TM} Red Mix (25ul, Bioline, London, UK), forward (ttr-4) and reverse (ttr-6) tetrathionate primers (20uM each), pure water (up to 50ul) and 200ng DNA template in a total volume of 50ul. A positive control (*S.* Typhimurium DT104), and negative control (pure water, no DNA template) was also used. PCRs were performed using Starlab (Milton Keynes, UK) wells closed with Starlab caps. Samples were run using

the Applied Biosystem 0.2720 Thermal Cycler machine. The conditions for the reactions were 95°C for 3 minutes for initial denaturation, 30s at 95°C for denaturation. 30s at 55°C for annealing to occur, followed by a 30 second extension phase at 72°C repeated for 35 cycles.

2.9 Whole genome sequencing

The pathway by which a decision was made as to whether samples were submitted for WGS is laid out in Figure 4 and 5; Results. Samples were submitted for WGS to the Earlham Laboratory, Norwich as part of the 10,000 *Salmonella* Genome Project (http://10k-salmonella-genomes.com, last accessed 26th November 2017). DNA extraction preparation for sequencing was carried out by the Earlham Laboratory. Illumina short-read sequencing was carried out on these samples. In total 259 isolates were submitted for whole genome sequencing.

3.0 Analysis of results

The database Enterobase was used to analyse results of the 72 *S. enterica* genomes from which sequencing had been completed at the time of writing. The accession numbers and further information of these isolates can be found in the results and on Enterobase (https://enterobase.warwick.ac.uk, last accessed 5th January 2018). Enterobase is a free, online tool for visualising genetic variation within enteric bacteria, providing assembled genomes and associated metadata on strain properties for public access. Genomes have been assembled *de novo* from Illumina reads from the Earlham Laboratory and genotyping data has been deduced exclusively from the assemblies using this programme. The sequence types, and subsequent serovars, have been deduced using the Enterobase program. The accession numbers of the 72 positive samples are provided in the results section.

3.1 Statistical Analysis

Descriptive statistics with a 95% confidence interval were used to describe the prevalence and diversity of NTS detected. The frequency and diversity of the antimicrobial susceptibility phenotypes of the NTS detected were also analysed using descriptive statistics with a 95% confidence interval. Prevalences discussed here were compared only visually and graphically, no further statistical tests have been used to analyse the data from this study.

Case definition:

Final case definition is any isolate confirmed to be NTS by whole genome sequencing. Those isolates which appear to be NTS following selective culture and antigen serotyping but results of whole genome sequencing have not yet been returned were not be counted as a case of NTS.

Chapter 3: Results

Introduction and Main Findings

I) Prevalence, diversity and strains of NTS

A variety of zoonotic serovars of NTS were detected in faecal and mesenteric lymph nodes of pigs at each site, including *S.* Typhimurium and *S.* Enteritidis. One isolate of *S.* Typhimurium ST313 was identified in the mesenteric lymph node of a pig slaughtered in Nairobi, Kenya and four unique isolates of *S.* Typhimurium ST19 were detected in pigs at slaughter in Nairobi. Unfortunately, it is currently impossible to offer an accurate prevalence of NTS in samples submitted from the three study sites as only partial whole genome sequencing results from each of the three sites have yet to be returned, therefore definite confirmation, according to the case definition, that the isolates were NTS cannot be offered, rendering any current true prevalence inaccurate.

II) Antimicrobial resistance profile of NTS detected

Two multi-drug resistant (MDR) isolates (resistant to three or more classes of antibiotics) of NTS were detected in pigs slaughtered in Busia. 10% of current confirmed NTS isolates from Busia (4/39, CI 4-24%) and 14% of current confirmed NTS isolates from Nairobi (3/22, CI 5-3%) were resistant to 1-2 antimicrobials. A range of different antimicrobial susceptibility phenotypes were present, including resistance to the third-generation cephalosporin ceftazidime (1/61, 1.6%, CI 0.3-8.7% of total confirmed unique NTS isolates) and the fluoroquinolone, ciprofloxacin (2/61, 3.3%, CI 0.9-11.2% of total confirmed unique NTS isolates).

Study population

Mesenteric lymph node and faecal samples were taken from pigs following slaughter in three geographical areas (Figure 1). In total, 306 pigs were sampled in Ndumbuini slaughterhouse, Nairobi, 267 in Busia county and 65 pigs in Malawi. In total 638 pigs were sampled during the study period.

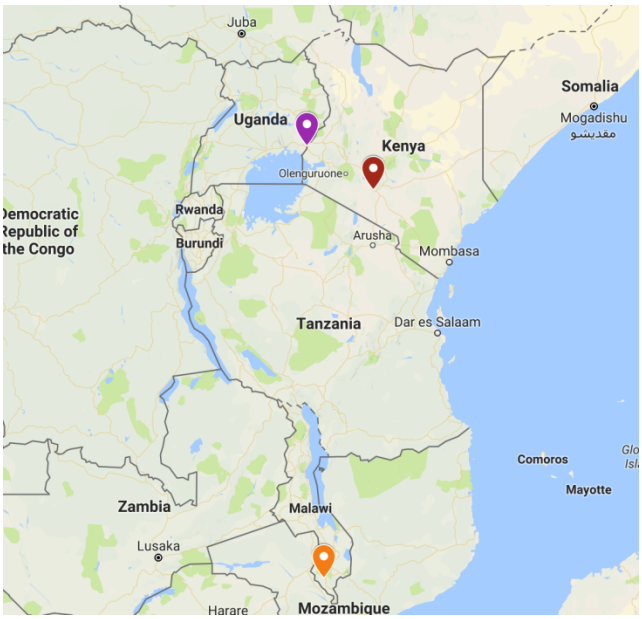


Figure 1. Location of the three study sites Nairobi and Busia, Kenya and Chikwawa Valley, Malawi

Sampling sites and locations pigs were reared

- Busia, Kenya

Up to 20 samples were collected daily over a six-week period October-November 2016 from seven slaughter slab locations across Busia county. Fewer than 20 pigs are slaughtered daily at each slab where samples were collected, and sampling from multiple sites daily fitted with the project schedule. The number of pigs sampled at each slaughter slab in Busia county is shown in Table 1.

Name of Slaughterslab in Busia County	Number of pigs sampled at this slaughterslab during this study	Percentage of total pigs sampled in Busia (%)
Mundika	124	46
Tanga kona	16	6
Nambale	28	10
Funyula	11	4
Matayos	35	13
Bumala	39	15
Mugatsi	7	3
Unknown slaughter slab within Busia county	7	3

Table 2. Number of pig samples collected from the slaughter slabs visited across Busia county during the study.

- Nairobi, Kenya

Nairobi samples were collected daily from up to 60 pigs from the Ndumbuini abbatoir (Figure 2a) over a two-week period November-December 2016. In total of 306 pigs were sampled. Pigs slaughtered in Ndumbuini slaughterhouse, Nairobi were reared in various geographical locations. The majority of pigs originated from the suburbs of Nairobi and the surrounds, but some had travelled from as far afield as Homa Bay, Kisumu, a distance of approximately 350km.

- Chikwawa District, Malawi

In Malawi up to 10 samples were collected daily over a two week period during April 2017, from butcheries and slaughter sites located in three towns in the Chikwawa District (Figure 2c); Nchalo, Ngabu and Chikwawa Town. In rural Malawi pigs are either slaughtered in the back yard of the owner or the slaughter man, prior to carcass processing in this location. It was found that meat was then transported uncontained, on foot or via a non-motorised bicycle, to the butchery. In Chikwawa Town samples were collected from the slaughter site of the pigs which was the back yard of the slaughter man. In Nchalo and Ngabu it was not possible to access the slaughter site and therefore four butcheries in Nchalo and two

butcheries in Ngabu were visited and sample collection was carried out directly at these butcheries.

Figure 2. Examples of slaughterhouses where samples were collected at each of the different study sites

a) Ndumbuini slaughterhouse, Nairobi

b) Bumala slaughterslab, Busia County

c) Chikwawa Town slaughterslab

Age of pigs

The age of the pigs sampled was estimated to range from 3 months to 2.5 years, with a mean of 14 months in Busia and 19 months in Malawi. This was unable to be defined in Nairobi as it was not possible to collect data regarding the age of the pigs, given the fast-paced slaughterhouse environment. In Nairobi samples were taken from 113 female pigs, 114 male pigs and 79 pigs of unknown sex. In Busia samples were taken from 146 females and 101 male pigs (10 of which were castrated) and 20 pigs of unknown sex. In Malawi samples were taken from 42 female pigs, 22 male pigs (5 of these were castrated) and one pig of unknown sex.

Breed of pigs

The most common breed sampled was a mixed breed white haired pig, likely a mix of the Landrace and large white breeds (Figure 3b). One hundred percent of the pigs sampled in Nairobi were of this breed and 30% of pigs in Busia. In Busia other breeds sampled included the Duroc (4%) and a local black breed in Kenya (19%) and a local black and white breed (20%) (Figure 3a). The breed of the remaining percentage of pigs was unknown. In Chikwawa, Malawi 98% of the study's pig population were the local black and white breed (Figure 3c), and 2% were unknown or unrecorded.

Figure 3. Examples of 'typical' local pig breeds sampled at each of the three study sites;

a) Busia

b) Nairobi

c) Malawi

<u>Transport of pigs to slaughter slabs</u>

In Nairobi pigs were mostly transported to the slaughterhouse singly or in small groups by pick-up truck. In Busia almost all pigs were transported to the slaughterhouse singly on a motorbike.

Previous antibiotic treatment

It was not possible to reliably determine whether previous antibiotic treatment had been administered to the pigs, as the animals were mostly brought to the slaughter slab or butchery by a Trader rather than an owner, and this Trader was not aware of the information. In the six cases where it was known that the pigs had been given antibiotics within the last 3 months, it was not known which antibiotic had been administered and at which dose and whether the owner of the pig had taken the drug's withdrawal period into account when choosing the day for slaughter. These six previously treated animals all originated from Busia. It was not possible to find out information regarding prior antibiotic treatment in either Nairobi or Malawi.

Total number of faecal and mesenteric lymph node samples collected from each location

Selective culture NTS was undertaken of both mesenteric lymph node and faecal samples collected from each pig in Busia, Nairobi and Malawi on both brilliant green and Harlequin ABC *Salmonella* agar. In occasional cases either a faecal or mesenteric lymph node sample from a pig was not collected and therefore only one sample was collected from each animal. The total numbers of samples collected in each sample location are detailed in Table 3 below.

Sample site and total number of pigs sampled	Number of pigs at each site from which faecal and mesenteric lymph node samples collected	Number of pigs at each site from which faecal sample only was collected	Number of pigs at each site from which mesenteric lymph node sample only was collected.
Busia county (N=267)	265	2	0
Nairobi (N=306)	306	0	0
Chikwawa District (N=65)	52	1	12

Table 3. Number of mesenteric lymph node and faecal samples collected and submitted for selective NTS culture at each location.

Non-typhoidal *Salmonella* detected

Prevalence of NTS in samples

As stated above, selective culture for NTS was undertaken of both mesenteric lymph node and faecal samples collected from each pig in Busia, Nairobi and Malawi on both brilliant green and Harlequin ABC *Salmonella* agar. NTS was detected by positive selective culture on either or both of Harlequin ABC *Salmonella* agar and brilliant green agar, O antigen serotyping and/or PCR. Positive isolates were submitted for whole genome sequencing (WGS) (see Figure 4 and 5). N.B. PCR was not undertaken on isolates collected from Malawi, therefore all isolates which gave a positive 'O antigen' serotype result from Malawi were submitted for whole genome sequencing; Figure 5)

Therefore, 110 positive NTS isolates were submitted for whole genome sequencing from pigs slaughtered in Nairobi, which originate from a total of 80 pigs. 57 NTS isolates were submitted from 38 pigs in the Chikwawa Valley, Malawi and 92 NTS isolates from Busia county were submitted from a total of 63 pigs. In total, 259 NTS isolates were submitted for whole genome sequencing at the Earlham Laboratory, Norwich, as part of the 10,000 *Salmonella* genome sequencing project (http://10k-salmonella-genomes.com)

Estimated Prevalence of NTS using positive isolates of selective culture, O antigen serotyping +/- PCR (see Table 3)

The estimated prevalence of NTS in pigs in Chikwawa (38/55, 58%, CI 46.5-70.4%) was found to be significantly higher than in pigs in Nairobi or Busia. There was a significantly lower prevalence of NTS detected in faecal samples of pigs in Busia (34/267, 12.7%, CI 8.7-16.7%), than the two other study locations and the estimated prevalence of NTS detected in mesenteric lymph node samples in Chikwawa (29/65, 44.6%, CI 33.1-57.5%) was significantly higher than that the other two other study sites. The estimated prevalence of NTS detected in both faecal and mesenteric lymph node samples from the same pigs was significantly higher in Chikwawa (7/38, 18.4%, CI 6.1-30.7%) than in Busia (6/63, 9.5%, CI 2.3-16.8%). The full results of the PCR have not been included in this thesis because they were not consistent with the culture and sensitivity results, nor the results of the whole genome sequencing which have currently been returned, therefore were not considered to provide accurate or reliable results. This was thought to occur as the PCR experiments were carried out under time pressure, as the first time that the operator had performed a PCR procedure.

Results of whole genome sequencing

To date, whole genome sequencing data has been returned from 159 isolates. These all originate from Kenya (65 from Busia, 94 from Nairobi). 72 (45.3% of the total submitted for whole genome sequencing) of these samples were found to be positive for NTS, the remainder were found to be bacterial isolates other than NTS.

Positive NTS isolates

46 of these positive NTS isolates originate from slaughterhouses in Busia and 26 from Nairobi. A table to show the positive NTS isolates currently confirmed on WGS is shown in the Appendix (Table I).

Duplicate NTS isolates

In total 20 'duplicate' isolates from Nairobi and Busia and 12 from Malawi were submitted for whole genome sequencing. A 'duplicate' in this case can be defined as two isolates from one pig which both originate from the same tissue source (eg either both from the same mesenteric lymph node sample or the same faecal sample) and which have cultured positive on both Harlequin ABC *Salmonella* agar and Brilliant agar, as well as both providing a positive 'O' antigen serotyping test and PCR result. A diagram to explain how this occurred using given the method is shown in Figure 4 below.

Figure 4. Sequence of methods used in Kenya.

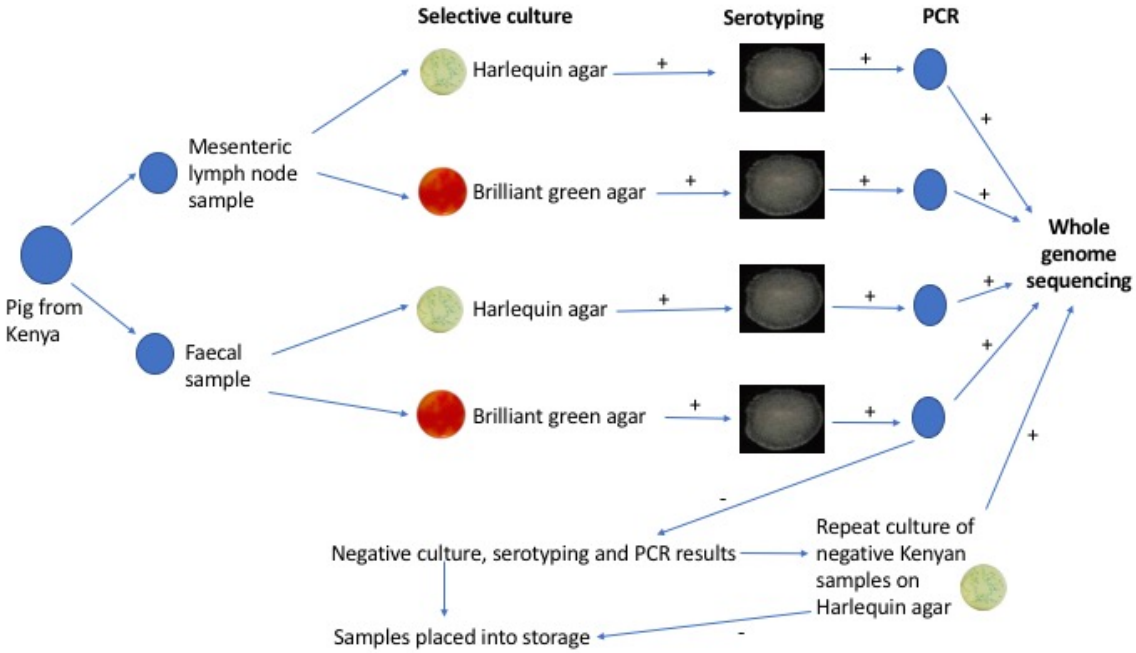
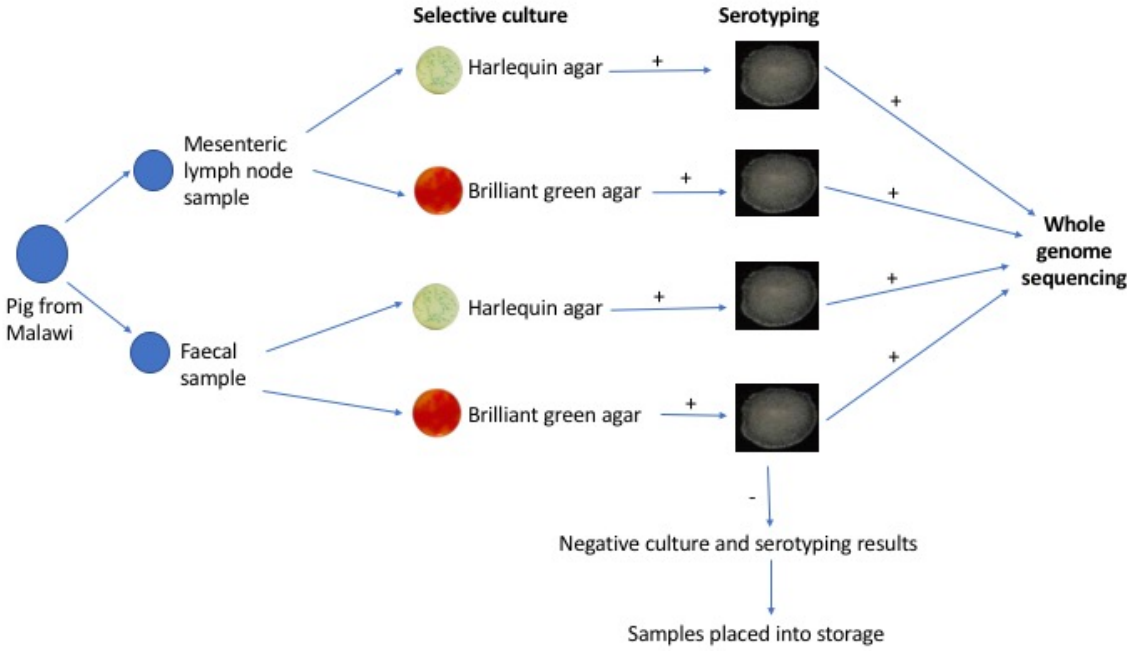


Figure 5. Sequence of methods used in Malawi



Of the 72 positive NTS genomes results received currently, 16 of these were from pigs which had 'duplicate' submissions to WGS, either from faecal or mesenteric lymph node samples. These 16 'duplicate' samples comprised of 12 from Busia and 4 from Nairobi (see Table I, Appendix). These duplicates were considered identical if the serovar, sequence type and antimicrobial resistance phenotype were shared exactly between the two isolates.

In 5 'duplicate' samples from Busia, although the serovar allocated by whole genome sequencing was the same between the duplicates, either the sequence type or antimicrobial susceptibility profile were different. This suggests that 'duplicate' isolates actually were unique and that the tissue source (either faecal or mesenteric lymph node from one pig) may be carrying two distinct NTS, as shown in Table 4 below.

Kenyan Number	Tissue sample originated from	NTS positive?	Serovar	Sequence type	Antimicrobial resistance profile
72	MLN HAC	Yes	Stanleyville	1986	Multi-drug resistant
72	MLN BGA	Yes	Stanleyville	912	Susceptible

Table 4. Illustrates the detection of 5 'non-identical duplicate' NTS, one of five examples from Busia. In this case although the NTS have been isolated from the same tissues and pig and the serovar is identical, the sequence type and antimicrobial resistance profile differ.

For the purpose of this thesis, those five salmonellae with identical serovars but differing sequence types and/or antimicrobial susceptibility profiles were therefore counted as unique isolates. Eleven duplicate isolates have currently been sequenced (7 from Busia and 4 from Nairobi). This is illustrated in Table 5 below.

Therefore 61 unique NTS isolates have completed whole genome sequencing; 39 from Busia and 22 from Nairobi (see Table 5 below).

Location	Total number of WGS results returned (N=72)	Character of isolate	Number
Busia	46	Duplicate isolates	7
		Unique isolates	39
Nairobi	26	Duplicate isolates	4
		Unique isolates	22

Table 5. Number of unique and duplicate isolates of NTS currently identified from each location.

Two isolates were found to be novel serovars of NTS previously unidentified by whole genome sequencing, both from pigs slaughtered in Nairobi (see Table I, Appendix).

Unique faecal and mesenteric lymph node isolates from Nairobi and Busia

Therefore, in total, from Nairobi 12 mesenteric lymph node NTS isolates and 10 faecal NTS isolates have been confirmed by whole genome sequencing. From Busia 11 faecal NTS isolates and 28 mesenteric lymph node NTS isolates have been confirmed.

Salmonella serovars identified

A variety of serovars of NTS have currently been identified in both locations, with some overlap of serovars between Nairobi and Busia. Table 6 demonstrates the isolates confirmed in each location by whole genome sequencing.

Busia (N=39)		**Both locations (N=22)**		**Nairobi (N=22)**	
Serovar	**Number of unique isolates confirmed**	**Serovar**	**Number of unique isolates confirmed**	**Serovar**	**Number of unique isolates confirmed**
Guildford	5	Fulica (Nairobi (4), Busia (7) Heidelberg (Nairobi (4), Busia (7))	11 11	Typhimurium	5
Uganda	3			Kiambu	1
Offa	2			Virchow	1
Enteritidis	1			Braederup	1
Newport	8			Anatum	1
Stanleyville	3			Muenchen	2
Tilene	1			Nairobi	1
Aberdeen	1			Previously untyped	2
Bovismorbificans	1				

Table 6. Demonstrating the different serovars of NTS confirmed to be isolated from pig by whole genome sequencing at each site. This includes unique isolates only, not duplicates.

Negative NTS isolates

As previously mentioned, whole genome sequencing data has currently been returned from 159 of the 259 isolates submitted for WGS. A full table of the isolates including serovar and sequence type of NTS as confirmed by whole genome sequencing in Nairobi and Busia, in addition to their antimicrobial resistance phenotypes as measured by antimicrobial

susceptibility testing, is shown in the Appendix (Table I). Of the 159 which were submitted for WGS results, 87 samples were found to be isolates other than NTS. 68 of these were from Nairobi and 19 from Busia. These bacteria comprised of a range of enteric commensal bacteria or bacteria found in the environment and include *Escherichia coli, Pseudomonas aeruginosa, Citrobacter freundii, Proteus mirabilis, Klebsiella pneumoniae, Moraxella osloensis, Comamonas testosteroni, Cronobacter sakazakii* and *Enterobacter cloacae,*

Pending whole genome sequencing results

The results of whole genome sequencing of 100 samples (57 from Malawi, 28 from Busia and 15 from Nairobi) are currently pending.

Sequence type of NTS isolates from each site

A variety of sequence types of *S. enterica* have been found on whole genome sequencing (Table I, Appendix). One isolate of *S.* Typhimurium ST313 and four unique isolates of *S.* Typhimurium ST19 have been isolated so far in a pig slaughtered in Ndumbuini slaughterhouse, Nairobi, Kenya. All of these isolates have susceptible antimicrobial resistance phenotypes, i.e. resistant to fewer than three classes of antibiotics.

Antimicrobial Susceptibility Phenotype of isolates

Currently the antimicrobial susceptibility phenotype only is available. Once the complete results of genome sequencing have returned it will be possible to make a comparison of the antimicrobial susceptibility phenotypes with the antimicrobial susceptibility genotypes of each positive isolate.

In this case 'susceptible' antimicrobial resistance phenotypes will be defined as those NTS isolates showing antimicrobial susceptibility phenotypes susceptible to all classes of antibiotics. Resistant isolates have been divided into those isolates susceptible to three or more antibiotic classes and those resistant to 1-2 classes of antibiotics. For the purpose of this thesis, the definition of a multi-drug resistance phenotype has been defined as 'a bacterial isolate resistant to three or more different classes of antibiotics'.

Here the antimicrobial susceptibility profile of unique isolates only, confirmed to be NTS by whole genome sequencing, have been considered. As no WGS results have currently returned from samples from Malawi, only these which have completed WGS from Busia and Nairobi are discussed here.

	Busia (N=39)(%, 95% CI)	**Nairobi (N=22) (%, 95% CI)**
MDR	2 (5, 1-17)	0
Resistant to 1-2 antibiotics	4 (10, 4-24)	3 (14, 5-33)
Susceptible to whole panel	32 (82, 67-91)	17 (77, 57-90)
Unmeasured	1 (3, 0-13)	2 (9, 3-28)

Table 7. Antimicrobial susceptibility profile of whole genome sequenced unique isolates from Nairobi and Busia.

Two multidrug resistant isolates were detected in samples from pigs from Busia (see Table 7). One from a faecal sample, serovar *S.* Fulica, resistant to ampicillin, chloramphenicol, ceftazidime, ciprofloxacin and cefotaxime, and the other serovar *S.* Stanleyville, isolated from a mesenteric lymph node with an AMR phenotype resistant to ampicillin, streptomycin, tetracycline, gentamicin and ciprofloxacin and cefotaxime and trimethoprim sulphamethoxazole (see Table 8). No NTS isolates from Nairobi which have undergone whole genome sequencing show multi-drug resistance (see Table 7).

Number of isolates	NTS serovar	Resistance Phenotype	Number of antimicrobial classes resistant to	Faecal or mesenteric lymph node sample	Location of sampling	% (95% CI) of antimicrobial resistance phenotype of the total number of unique whole genome sequenced samples from each location. Busia N=39 Nairobi N=22
1	Stanleyville	amp tet	2	MLN	Busia	3% (0-13%)
1	Stanleyville	amp strep tet gent cipro ctx trim	7	MLN	Busia	3% (0-13%)
1	Fulica	amp chlor caz cipro amoxy-clav	3	F	Busia	3% (0-13%)
1	Aberdeen	strep	1	MLN	Busia	3% (0-13%)
1	Bovismorbificans	tet	1	MLN	Busia	3% (0-13%)
1	Heidelberg	chlor ctx	2	MLN	Busia	3% (0-13%)
2	Typhimurium	strep tet	2	F	Nairobi	9% (3-28%)
				MLN	Nairobi	
1	Heidelberg	kana	1	F	Nairobi	5% (1-22%)

Table 8. The proportion of different resistant phenotypes in faecal and mesenteric lymph node samples confirmed as NTS by whole genome sequencing in Busia and Nairobi. Amp=ampicillin, tet=tetracycline, strep=streptomycin, gent=gentamicin, Cipro=ciprofloxacin, ctx=cefotaxime, trim=trimethoprim sulphamethoxazole, chlor=chloramphenicol, caz=ceftazidime, amoxy-clav=amoxicillin-clavulanic acid, kana=kanamycin

No important difference was found between the number of specific antimicrobial resistance susceptibility phenotypes measured by disc diffusion of those isolates fully confirmed NTS by whole genome sequencing from pigs slaughtered in Busia and Nairobi

(Table 8). Nine different antimicrobial susceptibility phenotypes were found, with only two of these, both *S.* Typhimurium were found to be resistant to the same antibiotics; streptomycin and tetracycline (see Table 8).

The percentage of the total unique isolates which have undergone whole genome sequencing showing an antimicrobial susceptibility phenotype resistant to each individual antibiotic is shown in Figure 6. From the isolates currently sequenced no significant difference has been found between the number of isolates resistant to each antibiotic and therefore currently no conclusion can currently be drawn as to which antibiotic or antibiotics show significantly higher phenotypic levels of resistance.

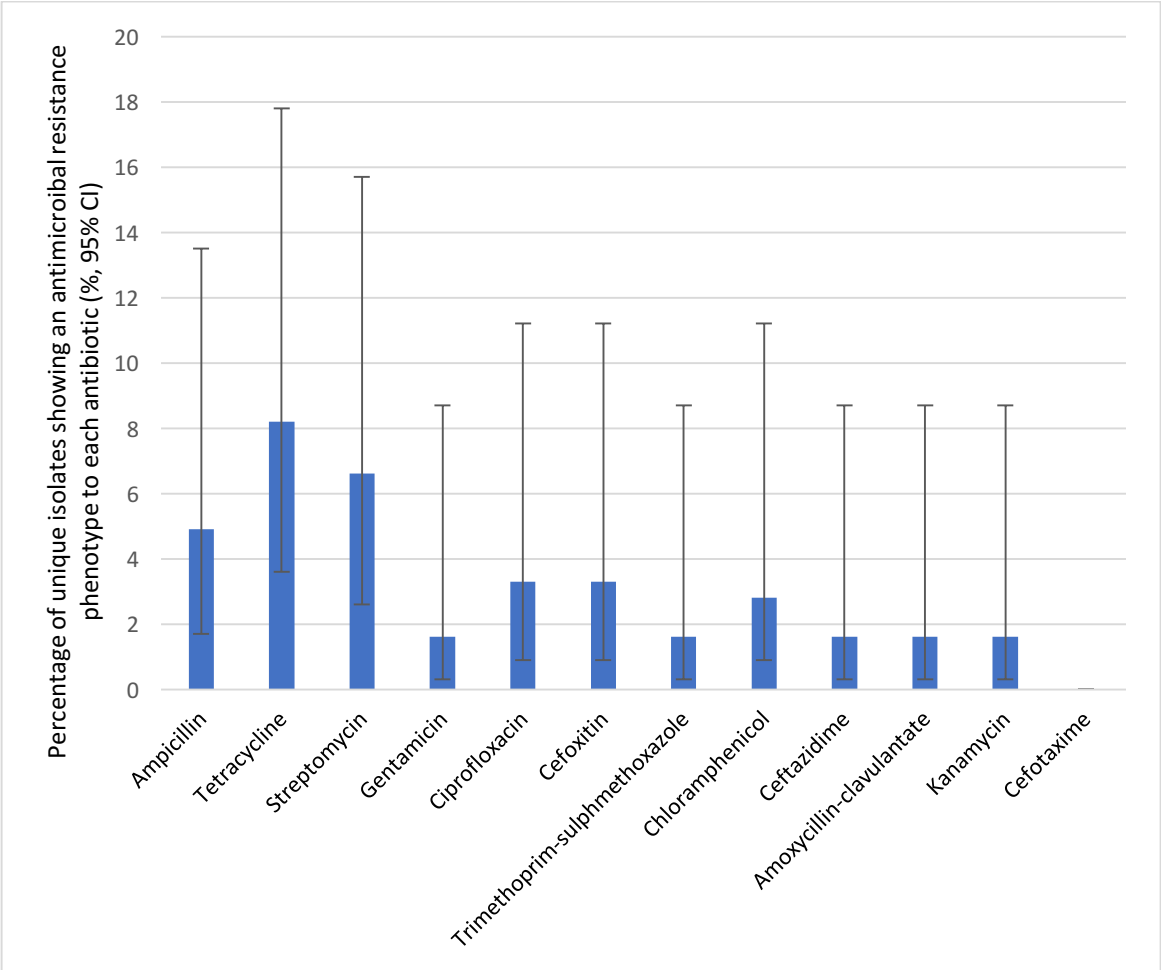


Figure 6. Percentage of total isolates from Busia and Nairobi exhibiting resistance to each antibiotic (N=61) (%, 95% CI)

Chapter 4: Discussion

Purpose of this study

The main aim of this thesis was to determine and compare the prevalence, strains and diversity of NTS in pig samples collected at post mortem from three slaughterhouse sites; two rural (Busia, Kenya and the Chikwawa Valley, Malawi) areas and one urban (Nairobi, Kenya) area. A secondary aim was to determine and compare the antimicrobial resistance profiles of the NTS present.

Results summary

i) Serovars and sequence types of NTS detected

The results showed that NTS is carried by pigs in the mesenteric lymph nodes in Kenya and Malawi and excreted into the environment in the faeces. A variety of different serovars were identified from pigs in Kenya, including *S.* Enteritidis, Virchow, Heidelberg, Fulica, Offa, Muenchen, Kiambu, Braenderup, Anatum, Guildford, Stanleyville, Newport, Uganda, Aberdeen, Bovismorbificans, and Tilene. Of particular concern was the discovery of *S.* Typhimurium with the sequence type ST313 in a mesenteric lymph node sample from a pig slaughtered in Ndumbuini abbatoir, Nairobi, Kenya and reared in Wangige area, about 15 minutes north of the Ndumbuini District. This sequence type has been found to be the cause of invasive salmonellosis in humans in sub-Saharan Africa (Kingsley, 2009). In total five unique isolates of *S.* Typhimurium were identified, all in pigs slaughtered in Nairobi, Kenya. In addition to the one *S.* Typhimurium ST313, the remaining four sequence types of isolates of this serovar were all ST19, which has been found to cause non-invasive diarrhoeal disease in humans worldwide (McClelland, 2001). The whole genome sequencing data of 159 of the 259 isolates submitted has currently been completed.

ii) Antimicrobial susceptibility phenotypes detected

The antimicrobial susceptibility phenotypes of the completed whole genome sequences demonstrated a variety of profiles, with resistance measured to a variety of the twelve drugs tested. Two multi-drug resistant isolates (resistant to more than three classes of antimicrobials) were detected amongst pigs in Busia, Kenya, both with differing antimicrobial susceptibility phenotypes. No isolates of NTS with MDR profiles were

detected from pigs in Nairobi. Interestingly, *S.* Typhimurium ST313 in humans in sub-Saharan Africa often carries a multi-drug resistant antimicrobial susceptibility phenotype, for example *S.* Typhimurium ST313 D23580 isolated in Malawi, which contains in its genome a composite genetic element encoding multi-drug resistant genes (Kingsley, 2009). The *S.* Typhimurium ST313 isolate detected in this study from a pig reared in Wangige, Nairobi was susceptible to the full range of antimicrobials tested on disc diffusion and further investigation to compare this susceptible and ST313 MDR genomes previously isolated from humans would be of extreme interest.

iii) Results applied in the current context

In the context of existing research, these results have demonstrated for the first time the presence of *S.* Typhimurium ST313 in pigs in sub-Saharan Africa. In a recent paper, Almeida et al. (2017) did find raw pork to be a source of *S.* Typhimurium ST313 in Brazil. In fact, this thesis reports the first instance that this sequence type has been discovered in any animal on the African continent, therefore the findings of this study are potentially extremely important. The discovery of ST313 in the mesenteric lymph node of a pig reveals the potential for an additional transmission route of this invasive sequence type of *S.* Typhimurium rather than solely human-to-human, as had been previously thought (Kariuki, 2006). Recent literature has leaned heavily towards the theory of a soley human-to-human model of transmission of in the invasive NTS bacteria; further adapted towards an invasive lifestyle in humans than other strains of NTS (Feasey, 2012, Okoro, 2012). This phenomenon has been characterised by genome degradation; Kingsley (2009) found that pseudogene formation and chromosomal deletions were present in the invasive *S.* Typhimurium ST313, in comparison to other non-invasive *S.* Typhimurium genome sequences, although as experimental infection of poultry with D23580 also leads to invasive infection, it may be more 'lifestyle' adapted than adapted to a specific host (Wigley, 2017). Therefore, the work of this thesis raises many further questions, such as whether the ST313 strain carried by this pig possesses the same genomic degradation and potentially invasive nature as that found in humans, and if not, whether any historical linkage of the human-isolated invasive strain and this pig-isolated strain can be established. This will be achieved by future in depth bioinformatics study of the pig-isolated *S.* Typhimurium ST313 genome.

Diarrhoeal and invasive disease caused by NTS serovars present a problem in the developed and developing world. From 2000-2008 NTS, largely driven by the serovars Typhimurium and Enteritidis, caused the highest burden of non-invasive gastrointestinal disease of all food-bourne pathogens in the United States of America; at 1,000,000 cases recorded per year with 380 annual deaths (Centers for Disease Control and Prevention, 2011). Most commonly in otherwise healthy humans, NTS causes self-limiting acute gastroenteritis, however, NTS disease is a major cause of invasive bacterial infection in humans in sub-Saharan Africa, particularly amongst the very young and old, and concurrently sick or immunocompromised humans of all ages. This invasive disease has mostly been found to be caused by the serovar *S.* Typhimurium, although *S.* Enteritidis does play a role, and together these serovars account for 91% of all invasive NTS recorded across sub-Saharan Africa (Uche, 2017, MacLennan, 2013, Feasey, 2012).

Generally, self-limiting gastroenteritis in humans caused by NTS is thought to be acquired by consumption of animal food products, especially undercooked meat, eggs and poultry (Mishu, 1994). Many serovars of NTS have a proven ability to cause diarrhoeal disease in humans often following a zoonotic transmission route, the most common of which is *S.* Typhimurium (Zhang, 2003). *S.* Typhimurium ST19 causes 'classical' and common gastroenteritis symptoms in humans across several continents, often associated with the strains LT2, SL1344 and DT104 NCTC13348 (Kingsley, 2009, McClelland, 2001, Okoro, 2012, Poppe, 1998). Carden (2003) found that *S.* Typhimurium ST19 often causes a greater degree of cellular invasion, macrophage death and consequent inflammatory reaction in the gastrointestinal tract than ST313. This correlates with the higher degree of gastrointestinal symptoms observed with *S.* Typhimurium ST19 infection in humans than *S.* Typhimurium ST313. The ability of *S.* Typhimurium ST19 to be transmitted zoonotically between animals and humans, particularly from poultry and pigs, has been discussed in numerous publications including Barrow (2012) and Parsons (2013). In this context, the finding of *S.* Typhimurium ST19 in faecal and mesenteric lymph node samples of pigs in Nairobi is of interest, and works to further confirm its importance in this context.

The antimicrobial susceptibility profiles of the NTS isolates found in this study also provide interesting results. Multidrug resistant isolates of NTS have been discovered in pigs in farming situations in higher income countries (Gebreves, 2005), however this thesis provides only the second description, to the author's knowledge, of the isolation of multi-drug resistant isolates of NTS from pigs in Africa (the first published by Kikuvi,

2007). The multidrug resistant phenotype found in the two sequenced isolates was not identical, and further work will be carried out in the future to investigate whether these antimicrobial resistance determinants lie within the core or accessory genomes of *S.* Typhimurium and therefore help to determine the potential for transfer of the antimicrobial resistance determinants between bacteria.

Kariuki (2002) found that NTS isolated from cattle, chickens and pigs close to the homes of index human hospitalised cases of NTS were more likely to be of a host-adapted, non-zoonotically transmitted serovar, susceptible to all antimicrobials tested. Previously a multi-drug resistant 100-110kb plasmid had been thought to transfer between chickens and humans between *S.* Typhimurium and *S.* Enteritidis on large scale commercial farms in Kenya (Kariuki, 1996). Further bioinformatics work to more closely examine the whole genome sequences of the *S.* Typhimurium detected in this study is needed to identify the genes associated with multi-drug resistance, as well as the location of these genes within the genome, particularly whether they are located on transferable, mobile genetic elements.

The hypothesis of this project was that invasive NTS was present in pigs suggesting the potential for an alternative transmission route for this disease from animals to humans, rather than solely human-to-human. The finding that pigs in sub-Saharan Africa are able to carry *S.* Typhimurium ST313 in their mesenteric lymph nodes raises the suspicion that zoonotic transmission may be possible. Furthermore, both serovars Enteritidis and Bovismorbificans were also isolated during this study, both of which have genetic variants associated with iNTS in Malawi (Bronowski, 2013, Feasey, 2016). However, more research needs to be carried out to prove this hypothesis such as concurrent faecal sampling of humans who were in contact with the slaughtered pigs during the weeks prior to slaughter as this provide stronger evidence to prove or disprove the theory of zoonotic transmission of iNTS in sub-Saharan Africa.

iv) Current status of results

At the current time, in total 259 isolates have been submitted to the Earlham Laboratories for whole genome sequencing, sequencing of 159 samples has been completed and sequencing data for positive NTS isolates has been returned from 72 of these samples. The remaining 87 samples were negative for NTS.

The project was conducted with the objective that NTS isolates would be confirmed to be NTS once the sequencing results had returned. All of the Malawi isolates have been submitted for sequencing based on a positive selective culture result and positive agglutination test with the Poly-O antigen alone (see Figure 5; Results). Of the 159 isolates for which the WGS results have already returned, 87 (54.7%) were found to be not NTS, or there was no growth within the samples. Given this high rate of negative isolates gained on WGS, and that samples submitted for whole genome sequencing have a 45.3% likelihood to be NTS, results of prevalence and the antimicrobial susceptibility profile currently are reported for confirmed isolates only.

v) Limitations of study design

Estimated sample size calculations had provided an estimate of appropriate sample size for the project to reach 154 pigs per study site. This figure was achieved in both Busia and Nairobi. However, due to delays in obtaining in country ethical approval for Malawi, the sampling time was reduced to a total of two weeks in this county and therefore only 65 pigs were sampled. Pigs are reared in a low intensity fashion in the Chikwawa Valley, where slaughter of pigs occurs infrequently according to demand. Fewer than five pigs were slaughtered in each village daily and it was therefore not logistically possible for the target sample size of 154 pigs to be met in this time period. Therefore, although the sequencing results for Malawi will be taken into consideration following their completion, this part of this study will not be adequately powered to establish significant conclusions using this data.

The sampling strategy in Malawi and Kenya presented challenges which could not have been forseen prior to the start of the project. In Malawi, particularly the Chikwawa Valley, the free-ranging pigs are slaughtered in the backyard of the owners, prior to transport of the exposed carcass on the back of a bicycle to the market butchery. This occurs as followers of the Islamic faith consider pigs to be impure animals and therefore the location of rearing and slaughter should be separate from that of cattle, sheep and goats. Although the Islam is followed by a minority of the population in Malawi, this cultural belief has influenced the practise of porcine slaughter in this country. This increased the difficulty of sample collection during this project. Animal Meat Inspectors (one working in each of the eleven Extension Planning Areas of the Chikwawa District) were on occasion able to be alerted to the location of slaughter of a pig in the hours prior to slaughter by

slaughter men, and were therefore able to alert the author that samples were ready to be collected. However, if this did not happen and the location of slaughter was unknown, sample collection relied on the owner or Trader of the pig remembering to bring the mesenteric lymph nodes and faecal sample to the butchery for collection. Although lessons in sampling technique were given to pig owners and Traders where possible prior to the onset of the study, it could not be guaranteed that samples were collected by the slaughter men hygienically and without contamination. In both study sites in Kenya sample collection was able to be undertake either by the author, or by trained fieldworkers supervised by the author, therefore in these cases hygienic and sanitary sample collection was able to take place, and contamination therefore minimised.

Should the study be extended or repeated, ethical permission to undertake faecal sample collection from human slaughterhouse workers or pig owners who are regularly in contact with pigs would be obtained and these samples would be collected simultaneously with the pig faecal sample collection. The lack of corresponding human data in this study means that the conclusion that serovars of NTS with zoonotic potential were affecting pigs is an assumption only, as there is no direct evidence that zoonotic transmission of disease has taken place at any of the study sites.

The antimicrobial susceptibility profiles of some isolates were incomplete. This was minimised as much as possible, but the antimicrobial disc for kanamycin was unavailable for testing in Busia. Therefore, an alternative aminoglycoside, gentamicin, was included in the disc diffusion protocol instead. Although gentamicin and kanamycin are both broad-spectrum antibiotics of the aminoglycoside family, it was not expected that there would be cross-over between the antimicrobial susceptibility phenotypes which the NTS found in the pigs displayed to these antibiotics.

Due to the cross-sectional nature of the project, sampling was carried out at the three study sites at different times of year which may have affected the results due to variations in seasonality. However, this was impossible to control for due to the scope of the project prohibiting repeat or simultaneous sampling at the study sites.

Collection of the faecal and mesenteric lymph node samples at the slaughterhouses, slaughter slabs and butcheries normally prohibited contact with the owner of the pigs. On the day of slaughter, pigs in Kenya and Malawi are bought from their original owner by a Trader, who takes the pigs to the assigned location where slaughter will take place.

Therefore, it was not possible to obtain exact GPS co-ordinates for the location where the pigs were reared, and precise details for metadata such as age of the pig and previous antimicrobial treatment were, in most cases, impossible to obtain as the Traders were not aware of this information. Had there been time and resources available, visits would have been paid to the location described by the Traders where the pigs had been reared and an interview with an owner of the pig would have been undertaken to ascertain more precise metadata.

'Duplicate' isolates in this project referred to isolates of NTS from the same tissue of the same pig detected on both modified brilliant green agar and Harlequin chromogenic agar (Lab M, Bury, UK), with identical serovars and sequence types as detected from analysis of the MLST results and AMR profiles. Some modified brilliant green agar and Harlequin chromogenic agar isolates from the same tissue of the same pig did present differences (e.g. Table 4; Results). This suggests that individual pigs in this study could potentially either be carrying multiple strains of NTS, and during this study two of these different strains were detected one on each of the agars. In this study, only one pick per plate of NTS was taken; to improve the reliability of this statement, multiple picks per plate would have been taken to confirm the diversity. Evidence of one pig carrying multiple strains of strains of NTS is also suggested by the presence of several pigs in the study with different isolates detected in the mesenteric lymph nodes and faeces. Further work in the future to determine the likelihood of both these situations would be of interest.

Pigs in the three study sites in sub-Saharan Africa carry a variety of potentially zoonotic NTS serovars in the mesenteric lymph nodes as well as excreting them in faeces. There is the potential for these zoonotic serovars to transmit to humans, either through fomites in the environment, or contamination of meat processing at slaughterhouses, butcheries or a household level. Therefore, pigs have the potential to transmit several serovars of NTS to and from humans.

vi) Antimicrobial Resistance

The most commonly used antimicrobials in humans in Kenya include doxycycline, tetracycline and co-trimoxazole as reported by Kariuki (Global Antimicrobial Resistance Partnership, 2011, taken from the importation licences and manufacturers' records at the Poisons and Pharmacy Board and the Ministry of Trade (Mitema, 2004)). Chloramphenicol usage was documented as high in humans as this drug was commonly

used to treat NTS and Typhoid fever. Fluoroquinolone and aminoglycoside use markedly increased between the period of 1997-1998 after the release of generic forms of the drug to the market (Mitema, 2004) and the emergence of resistance to chloramphenicol (Gordon, 2008). Mitema (2004) found that first generation cephalosporins were in greater use at this time than those of the second and third generation. Worryingly, half of medicines in Africa are used irrationally (Ecumenical Pharmaceutical Network, 2009) and antibiotics are freely available to treat both humans and animals without prescription, therefore patterns of dispensing for identical illnesses will vary, and may not always necessarily be appropriate or representative. The pricing of antibiotics may also play some role in the decision of which drug to use to treat an ill human or animal and in Kenya or Malawi it does not always follow that the broader spectrum antibiotics are the most expensive. Therefore, a patient or livestock owner's economic situation may, by necessity, influence the antibiotic treatment of choice to a greater degree than consideration of appropriate antimicrobial stewardship principles. In Kenya, it has previously been found that antibiotics such as the tetracyclines, penicillins, sulphonamides, gentamicin and chloramphenicol are dispensed by 70% of farmers without prescription in an unregulated manner for growth promotion, prophylaxis and treatment (Kariuki, 1997). However, practises may have changed over time since the publication of this paper. For example, from subjective observations, we are aware that currently streptomycin is used more often by farmers for their animals than gentamicin. It has been speculated that some farmers have been found to be more reliant on the use of antibiotics for raising good quality animals, rather than the implementation of proper feeding practices or husbandry protocols. Adelaide (2008) detected a high percentage of antimicrobial resistant *E. coli* isolates in chickens at a processing plant in Limuru, Kenya; 75.9% of isolates showed an antimicrobial resistance phenotype resistant to tetracycline, 39% to ampicillin, 13.2% to chloramphenicol, 72.4% to co-trimoxazole and 19% to ciprofloxacin where antibiotics were regularly used as prophylaxis. Kikuvi (2007) detected 35.7% antimicrobial resistance from a small sample size of 16 NTS positive pigs. These NTS isolates showed various AMR phenotypes, including antimicrobial resistance to ampicillin, chloramphenicol, streptomycin and tetracycline, encoded by the genes *catAI, strA* and *tet(A),* respectively.

In this study, although there was minimal difference between the percentage of the unique isolates detected in Kenya, which show antimicrobial resistance to different antibiotics, ampicillin (4.9%, CI 1.7-13.5), tetracycline (8.2%, CI 3.6-17.8) and streptomycin

(6.6%, CI 2.6-15.7) showed a higher percentage of resistance than the other nine drugs included in the panel. This correlates with the high percentage resistance to these drugs detected in chickens in Limuru (Adelaide, 2008) and the resistant antibiotic phenotypes detected by Kikuvi's study (2007).

Musicha (2017) has published surveillance data of antibiotic resistance as detected in the Queen Elizabeth Central Hospital, Blantyre, Malawi from 1998-2016. 194,539 blood culture samples were tested and 29,183 pathogens were isolated over this time period. 51.1% of these were resistant to the first-line antibiotics used in Malawi; penicillin, chloramphenicol and co-trimoxazole. The proportion of non-*Salmonellae Enterobacteriaceae* with resistance to third generation cephalosporins and fluoroquinolones rose throughout the study period to 61.9% in 2016. This is an extremely high level of resistance and close attention will be paid to the AMR profiles of any positive NTS isolates in pigs from Malawi to detect whether there is correlation between the human and animal data.

vii) Implications of this study

Knowledge of the diverse range of zoonotic serovars of NTS carried and excreted by pigs in the study sites raises particular concern for the potential risk which pigs may pose in the zoonotic transmission of this bacteria. Pigs in rural areas of Kenya and Malawi are free roaming and so have access to human faeces to eat, as well as the potential to defecate within easy access of humans, including children. Pigs reared in more commercial, urban environments, such as some of the animals slaughtered at Ndumbuini slaughterhouse, Nairobi, also live in close proximity, therefore the frequent contact between the animals within a herd increases the risk of transmission of these bacteria between them.

Zoonotic transmission of the bacteria to humans, depending on the serovar, sequence type and strain, may lead to mild, self-limiting gastroenteritis, as reported in several studies, caused by serovars discovered in faeces or mesenteric lymph nodes of pigs in this study, or potentially more invasive, debilitating disease, particularly amongst those who are concurrently ill or immune compromised. Invasive disease can be caused in humans by sequence type ST313, found in humans in sub-Saharan Africa, and never previously identified in pigs on this continent. The identification in this study of one *S.* Typhimurium ST313 strain in the mesenteric lymph node in a pig slaughtered in Ndumbuini

slaughterhouse, Nairobi, raises concerns that pigs may be implicated in the zoonotic spread of this bacteria, and further work needs to be performed to confirm this.

Practical applications of the results of this study differ varying on the environment in which the pigs are reared. In some counties and districts in Kenya and Malawi, free range pig production has been outlawed. This is not the case in Busia County or the Chikwawa Valley. Implementation of this by-law would limit the access of pigs to human faeces for consumption of waste contaminated with NTS serovars, and limit the contact of humans to porcine faeces, the disposal of which could be more hygienically managed in a more contained environment. Hygiene considerations should also be reviewed in more intensive pig-rearing facilities in Kenya, from which some of the samples from Ndumboini slaughterhouse originated. This would include appropriate protection and personal hygiene, such as washing hands for the stockmen handling the pigs, and a clear hygiene and sanitation policy and protocol for controlling the mixing of pigs. The aim of this would be to limit the spread of potentially zoonotic bacteria and transfer of antimicrobial resistance determinants.

viii) Recommendations for further research

The results of this study will be expanded once all isolates submitted for whole genome sequencing have completed their processing. This will enable a full picture of the prevalence of NTS and diversity of serovars, sequence types and antimicrobial susceptibility genotypes to be ascertained. The current results provide baseline data of the more prevalent serovars of NTS detected in the pigs.

There are numerous extensions of this study which would be of extreme interest. A natural subsequent study would involve collecting retrospective human data of previously identified NTS infections from the same area that the pigs were reared or slaughtered in order to make a comparison between the nature of the NTS isolated from pigs during this study and that of humans in the same area. A further extension would be sampling the stools of pigs and their owners, farm workers and the slaughterhouse workers simultaneously. This would help to ascertain whether both humans and pigs carry identical isolates of NTS, and so investigate the potential for zoonotic transmission in these environments in Kenya and Malawi. Surveillance of febrile or diarrhoeic patients admitted to hospital with confirmed NTS infection would also be informative. Sampling of in-contact humans and animals at the household of the patient, to ascertain whether

human-to-human or zoonotic transmission had occurred, would provide evidence to determine whether transmission of NTS by pigs or other in contact animals at a household level had the potential to lead to clinical disease in humans.

Antimicrobial usage data for both humans and pigs from areas where pigs were reared and slaughtered in this study would have been useful to put the results into context. In this survey, the Traders who brought the pigs to the slaughterhouse were unaware of previous treatment which had been administered to the pigs, either name of drug, dose or frequency, and this information would have been extremely useful to assess previous exposure of the pigs to antimicrobial drugs. From subjective observations, we know that the most commonly used antimicrobials in the study sites are generally ampicillin, tetracycline and streptomycin, and this is realised in the results seen in this project as these are the most common antimicrobials for which resistance was found in the NTS isolates cultured. This suggests that there is transfer of antimicrobial resistance determinants at some level of the population between the pigs and the humans, although the exact route of this transfer is not yet known. Further study of interactions between the species at a household and village level would help us to understand to the movement of antimicrobial resistance determinants through a population, and therefore assist in the limiting this spread. If, as the Traders mostly suspected on questioning, antimicrobial treatment in pigs is a rare event, the antimicrobial resistance determinants present in the NTS carried and excreted by the pigs must either arise by spontaneous mutation, or more likely having been acquired from an outside source, therefore it would be extremely useful to determine whence-forth these determinants came. One other factor which would be interesting to assess is the change in antimicrobial susceptibility patterns in NTS isolated from pigs in the field over time. However, this study would present challenges to perform in field conditions, owing to the low intensity nature of pig farming in both Busia and Malawi, and the difficulty in repeat sampling of identical pigs in the field.

ix) Conclusions

This study shows that pigs at slaughter in Busia and Nairobi, Kenya and the Chikwawa Valley, Malawi carry a variety of NTS serovars with assorted AMR profiles, including MDR phenotypes. There is the potential for zoonotic transmission of NTS between pigs and humans in Kenya and Malawi and the transfer of AMR determinants between these species should be considered.

References

Adelaide O, Bii C, Okemo P (2008) Antibiotic resistance and virulence factors in Escherichia coli from broiler chicken slaughtered at Tigoni processing plant in Limuru, *Kenya East Afr Med J* 85(12):597-606

Agustin A, Carraminana J, Rota C, Herrera A (2005) Antimicrobial resistance of *Salmonella spp*. From pigs at slaughter in Spain in 1993 and 2001 *Lett. Appl. Microbiol.* 41:39-44

AHVLA (2014) *Salmonella* in livestock production in GB: 2013 report. Available from: http://www.defra.gov.uk/ahvla-en/publicaions/salm13/. Last accessed 21 August 2017

Alban L, Stege H, Dahl j (2002) The new classified system for slaughter pig herds in the Danish *Salmonella* surveillance-and-control program. *Prev Vet Med* 53:133-46

Alban L, Baptista FM, Mogelmose v, Sorensen ll, Christensen H, Aabo S, Dahl J (2012) *Salmonella* surveillance and control for finisher pigs and pork in Denmark- a case study. *Food Res Intl* 45: 656-65

Almeida F, Aparecida Seribellia A, Silva p, Ines M, Medeiros C, Prazeres Rodrigues D, Gallina Moreira C, Allard M, Pfrimer Falcao J (2017) Multilocus sequence typing of *Salmonella* Typhimurium reveals the presence of the highly invasive ST313 in Brazil *Inf Gen and Evol* 51(68) 41-44

Amaechi, N., and Ezeronye OU (2006) Piggery environment as a source of *Salmonella* contamination for swine*. J. Anim. Vet. Adv.* 5, 102–107.

Amass SF (2005a) Biosecurity: stopping the bugs from getting in. *Pig J* 55:104-14

Andres V, Davies R (2015) Biosecurity measures to control *Salmonella* and other infectious agents in pig farms: A review *Comp Rev Food Sci and Food Safety* 14:4 317-335

Ao TT, Feasey NA, Gordon MA, Keddy KH, Angulo FJ, Crump JA (2015) Global burden of invasive non-typhoidal *Salmonella* disease, 2010. *Emerg Infect Dis* 21(6), 941-949

Arcangioli MA, Leroy-Setrin S, Martel JL, Chaslus-Dancla E (2000) Evolution of chloramphenicol resistance, with emergence of cross-resistance to florfenicol, in bovine *Salmonella* Typhimurium strains implicates definitive phage type (DT) 104. *J Med Microbiol* 49:103–110.

Arguello H, Alvarez-Ordonez A, Carvajal A, Rubio P, Prieto M (2013) Role of slaughtering in Salmonella spreading and control in pork production *J Food Prot* 76(5):889-911

Arthur G, Nduba VN, Kariuki SM, Kimari J, Bhatt SM, Gilks CF (2001) Trends in bloodstream infections among human immunodeficiency virus-infected adults admitted to a hospital in Nairobi, Kenya, during the last decade. *Clin Infect Dis* 33:248 –256.

Barcelo J, Marco E (1998) On farm biosecurity. Proceedings of the 15th Intl Pig Vet Society Congress July 5-9, Birmingham, England: Nottingham Univ. Press

Barrow PA, Jones MA, Smith AL, Wigley P (2012) The long view: Salmonella– the last forty years. Avian pathology: Journal of the WVPA 41: 413–420

Bearson B, Brunelle B (2015) Fluoroquinolone induction of phage-mediated gene transfer in multidrug-resistant *Salmonella Int Jour Antimicrobial Agents* 46(2) 201-204

Behl P, Gupta V, Sachdev A, Guglani V Chander J (2017) Patterns in antimicrobial susceptibility of *Salmonellae* isolated at a tertiary care hospital in northern *India Indian J Med Res* 145(1): 124-128

Ben Hassen A, Bejaoui M, Lakhoua MR, Ben Redjeb S. (1993) Epidemiological pattern of the resistance of 153 *Salmonella* strains (S. Typhi excluded) isolated in a Tunisian pediatric unit from 1985 to 1990. *Pathol Biol (Paris)* 41:706 –712.

Benacer D, Thong KL, Watanabe H, Puthucheary SD. (2010) Characterization of drug resistant *Salmonella* enterica serotype Typhimurium by antibiograms, plasmids, integrons, resistance genes and PFGE. *J Microbiol Biotechnol* 20:1042–1052

Berends BR, Urlings HA, Snijders JM, Van Knapen F (1996) Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. *Int J Food Microbiol*. 30:37–53

Berkley JA, Lowe BS, Mwangi I, Williams T, Banui E, Mwarumba S, Ngetsa C, Slack MPE, Njenga S, Hart CA, Maitland K, English M, Marsh K, Scott JAG. (2005) Bacteraemia among children admitted to a rural hospital in Kenya. *N Engl J Med* 352:39 –47.

Berkley JA, Bejon P, Mwangi T, Gwer S, Maitland K, Williams TN, Mohammed S, Osier F, Kinyanjui S, Fegan G, Lowe BS, English M, Peshu N, Marsh K, Newton CRJC. (2009) HIV infection, malnutrition, and invasive bacterial infection among children with severe malaria. *Clin Infect Dis* 49:336 –343.

Biggs HM, Lester R, Nadjm B, Mtove G, Todd JE, Kinabo GD, Philemon R, Amos B, Morrissey AB, Reyburn H, Crump JA. (2014) Invasive *Salmonella* infections in areas of high and low malaria transmission intensity in Tanzania. *Clin Infect Dis* 58:638 –647.

Boyd D, Cloeckaert A, Chaslus-Dancla E, Mulvey MR. (2002) Characterization of variant *Salmonella* genomic island 1 multidrug resistance regions from serovars Typhimurium DT104 and Agona. *Antimicrob Agents Chemother* 46:1714 –1722.

Boyen F, Hasebrouck F, Maes D, van Innerseel F, Ducatelle R, Pasmans F (2008) Non-typhoidal *Salmonella* infections in pigs: A closer look at epidemiology, pathogenesis and control. *Vet Microbiol* 130:1-19

Brent AJ, Oundo JO, Mwangi I, Ochola L, Lowe B, Berkley JA. (2006) *Salmonella* bacteremia in Kenyan children. *Pediatr Infect Dis J* 25:230 – 236

Bronowski C, Fookes M, Gilderthorp R, Ashelford K, Harris S, Phiri A, Hall N, Gordon M, Wain J, Hart C, Wigley P, Thomsom N, Winstanley C (2013) Genomic characterisation of invasive non-typhoidal *Salmonella enterica* subspecies *enterica* serovar bovismorbificans isolates from Malawi *PLoS Negl Trop Dis* 7(11): e2557

Bronzan RN, Taylor TE, Mwenechanya J, Tembo M, Kayira K, Bwanaisa L, Njobvu A, Kondowe W, Chalira C, Walsh AL, Phiri A, Wilson LK, Molyneux ME, Graham SM. (2007) Bacteremia in Malawian children with severe malaria: prevalence, etiology, HIV co-infection, and outcome. *J Infect Dis* 195:895–904.

Butaye P, Michael GB, Schwarz S, Barrett TJ, Brisabois A, White DG (2006) The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. *Microbes Infect* 8:1891–1897.

Carden S, Okoro C, Dougan G, & Monack D (2015) Non-typhoidal *Salmonella* Typhimurium ST313 isolates that cause bacteremia in humans stimulate less inflammasome activation than ST19 isolates associated with gastroenteritis. *Pathogens and Disease*, *73*(4)

Caudron JM, Ford N, Henkens M, Macé C, Kiddle-Monroe R, Pinel J. (2008) Substandard medicines in resource-poor settings: a problem that can no longer be ignored. *Trop Med Int Health* 13:1062–1072

Centers for Disease Control and Prevention. (2011) Compendium of measures to prevent disease associated with animals in public settings, 2011. MMWR *Morb Mortal Wkly Rep* 60:1–24.

Choudhary A, Gopalakrishnan R, Senthur N, Ramasubramanian V, Ghafur K, Thirunarayan M (2013) Antimicrobial susceptibility of Salmonella enterica serovars in a tertiary care hospital in southern India *The Indian Journal of Medical Research* 137(4), 800-802

Cirillo DM, Valdivia RH, Monack DM, Falkow S (1998) Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. *Mol Microbiol* 30: 175–188

Clottey SJA (1985) Manual for the slaughter of small ruminant in developing countries Rome: Food and Agriculture Organisation of the United Nations

Codex-Alimentaruis-Commission (2005) Code of Hygenic Practice for Meat. Rome: Food and Agricultural Organisation of the United Nations.

Cook EAJ, de Glanville WA, Thomas LF, Kariuki S, Bronsvoort BMC, Fevre E (2017) Working conditions and public health risks in slaughterhouses in western Kenya BMC Public Health 17:14

Crump JA, Medalla FM, Joyce KW, Krueger AL, Hoekstra RM, Whichard JM, Barzilay EJ, Emerging Infections Program NARMS Working Group. (2011) Antimicrobial resistance among invasive non-typhoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrob Agents Chemother* 55:1148 –1154

Crump J, Sjolund-Karlsson M, Gordon M, Parry C (2015) Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections *Clin Microbiol Review* 28(4) pp910-937

Cunningham SA, Sloan LM, Nyre LM, et al. (2010) Three-hour molecular detection of *Campylobacter, Salmonella, Yersinia,* and *Shigella* species in feces with accuracy as high as that of culture. *J Clin Microbiol* 48:2929–2933.

de la Cruz ML, Conrado I, Nault, Perez A, Dominguez L, Alvarez J (2017) Vaccination as a control strategy against Salmonella infection in pigs: A systemic review and meta-analysis of the literature *Res Vet Sci*

Deen J, von Seidlein L, Andersen F, Elle N, White N, Lubell Y (2012) Community-acquired bacterial bloodstream infections in developing countries in south and southeast Asia: a systematic review. *Lancet Infect Dis* 12:480 –487

Delarocque-Astagneau E, Bouillant C, Vaillant V, Bouvet P, Grimont PA, Desenclos JC (2000) Risk factors for the occurrence of sporadic *Salmonella enterica* serotype Typhimurium infections in children in France: a national case-control study. *Clin Infect Dis* 31:488 –492

Denagamage T, O'Connor A, Sargeant J, Rajic A, McKean J (2007) Efficacy of vaccination to reduce Salmonella prevalence in live and slaughtered swine: A systematic review of literature from 1979 to 2007 *Foodbourn Path and Dis* 4(4):539-549

Doublet B, Boyd D, Mulvey MR, Cloeckaert A. (2005) The *Salmonella* genomic island 1 is an integrative mobilizable element. *Mol Microbiol* 55:1911–1924.

Ecumenical Pharmaceutical Network (2009) Fight AMR! Call to Action. Pharmaceutical Network Nairobi

EFSA (2008) Report of the task force on zoonoses data collection on the analysis of the baseline survey on the prevalence of *Salmonella* in slaughter pigs, Part A: *Salmonella* prevalence estimates. *EFSA J.* 6, 111–pp.

EFSA (2009) Analysis of the baseline survey on the prevalence of *Salmonella* in holdings with breeding pigs in the EU, 2008- Part A: *Salmonella* prevalence estimates *EFSA J* 7(12): 1377, 1-93

Enwere G, Biney E, Cheung YB, et al. (2006) Epidemiologic and clinical characteristics of community-acquired invasive bacterial infections in children aged 2–29 months in The Gambia. *Pediatr Infect Dis J* 25:700–5.

EUCAST (2017) Available at: http://www.EUCAST.org. Last accessed 1.9.17

FAO Abattoir Development (2010) In: Options and designs for hygenic basic and medium sized abbatoirs Rome: Food and Agricultural Organisation for the United Nations

F.C.C. Consortium (2010) Analysis of the costs and benefits of setting a target for the reduction of *Salmonella* in slaughtered pigs. Report for European Commission Health and Consumers Directorate General, Available at: http://ec.europa.eu/food/food/biosafetly/salmonella/docs/fattening_pigs_analysis_costs.pdf

Feasey N, Archer B, Heyderman R (2010) Typhoid fever and invasive non-typhoidal salmonellosis, Malawi and South Africa. *Emerg Infect Dis* 2010; 16:1448–1451.

Feasey N, Dougan G, Kingsley R, Heyderman R, Gordon M (2012) Invasive non-typhoidal *Salmonella* disease: an emerging and neglected tropical disease in Africa. *Lancet* 379:2489 – 2499.

Feasey N, Houston A, Mukaka M, Komrower D, Mwalukomo T, Tenthani L, Jahn A, Moore M, Peters R, Gordon M, Everett D, French N, van Oosterhout J, Allain T, Heyderman R (2014) A reduction in adult blood stream infection and case fatality at a large African hospital following antiretroviral therapy roll-out. *PLoS One* 9: e92226

Feasey N, Masesa C, Jassi C, Faragher E, Mallewa J, Mallewa M, MacLennan CA, Msefula C, Heyderman R, Gordon M (2015) Three epidemics of invasive multidrug-resistant *Salmonella* bloodstream infection in Blantyre, Malawi, 1998-2014 *Clin Infect Dis* 1:62 Suppl 4:S363-71

Feasey N, Hadfield J, Keddy K, Dallman T, Jacobs J, Dend X, Wigley P, Barquist L, Langridge G, Feltwell T, Harris S, Mather A, Fookes M, Aslett M, Msefula C, Kariuki S, Maclennan CA, Onsare RS, Weill FX, Le Hello S, Smith AM, McClelland M, Desai P, Parry CM, Cheesbrough J, French N, Campos J, Chabalgoity JA, Betancor L, Hopkins KL, Nair S, Humphrey TJ, Lunguva O, Cogan TA, Tapia MD, Sow SO, Tennant SM, Bornstein K, Levine MM, Lacharme-Lora L, Everett DB, Kingsley RA, Parkhill J, Heyderman RS, Dougan G, Gordon MA, Thomson NR (2016) Distinct *Salmonella Enteritidis* lineages associated with enterocolitis in high-income settings and invasive disease in low-income settings *Nat Genet* 48(10):1217-1217

Funk J, Davies P, Gebreyes W (2001) Risk factors associated with Salmonella enterica prevalence in three-site swine production systems in North Carolina, USA *Berl Munch Tierarztl Wochenschr* 114(9-10):335-8

Gal-Mor O, Boyle E., Grassi G. (2014) Same species, different diseases, how and why typhoidal and non-typhoidal *Salmonella enterica* serovars differ *Front Microbiol* 5 pp391-321

Gebreyes W, Davies A, Morrow W, Funk J, Altier C (2000) Antimicrobial resistance of *Salmonella* isolates from swine *J. Clin Microbiol* 38:4633-4636

Gebreves W, Thakur S (2005) Multidrug-resistant *Salmonella enterica* Serovar Muenchen from pigs and humans and potential inter-serovar transfer of antimicrobial resistance *Antimicrob Agents Chemotherap* 49(2): 503-511

Gebreyes W, Thakur S, Dorr P, Tadesse D, Post K, Wolf L (2009) Occurrence of spvA virulence gene and clinical significance for multi-drug resistant Salmonella strains *Jour Clin Micro* 47(3) 777-780

Giammanco GM, Pignato S, Mammina C, Grimont F, Grimont PA, et al. (2002) Persistent endemicity of *Salmonella bongori* 48:z(35):--in Southern Italy: molecular characterization of human, animal, and environmental isolates. *J Clin Microbiol.* 40:3502–3505

Gilks CF, Brindle RJ, Otieno LS, Simani PM, Newnham RS, Bhatt SM, Lule GN, Okelo GBA, Watkins WM, Waiyaki PG, Were JBO, Warrell DA (1990) Life-threatening bacteremia in HIV-1 seropositive adults admitted to hospital in Nairobi, Kenya. *Lancet* 336:545–549

Glenn LM, Lindsey RL, Frank JF, Meinersmann RJ, Englen MD, Fedorka-Cray PJ, Frye JG (2011) Analysis of antimicrobial resistance genes detected in multidrug-resistant *Salmonella enterica* serovar Typhimurium isolated from food animals. *Microb Drug Resist* 17:407–418

Global Antibiotic Resistance Partnership—Kenya Working Group (2011) Situation Analysis and Recommendations: Antibiotic Use and Resistance in Kenya. Washington, DC and New Delhi: Center for Disease Dynamics, Economics & Policy

Gondwe EN, Molyneux ME, Goodall M, Graham SM, Mastroeni P Drayson MT, MacLennan CA (2010) Importance of antibody and complement for oxidative burst and killing of invasive non-typhoidal *Salmonella* by blood cells in Africans. *Proc Natl Acad Sci U S A* 107:3070 –3075

Gordon MA, Walsh AL, Chaponda M, Soko D, Mbvwinji M, Molyneux ME, Gordon SB (2001) Bacteremia and mortality among adult medical admissions in Malawi: predominance of non-Typhi salmonellae and *Streptococcus pneumoniae. J Infect* 42:44 –49.

Gordon MA, Banda HT, Gondwe M, Gordon SB, Boeree MJ, Walsh AL, Corkill JE, Hart CA, Gilks CF, Molyneux ME (2002) Non-typhoidal *Salmonella* bacteraemia among HIV-infected Malawian adults: high mortality and frequent recrudescence. *AIDS* 16:1633–1641.

Gordon MA, Gordon SB, Musaya L, Zijlstra EE, Molyneux ME, Read RC (2007) Primary macrophages from HIV-infected adults show dysregulated cytokine responses to *Salmonella*, but normal internalization and killing. *AIDS* 21:2399 –2408.

Gordon MA, Graham SM, Walsh AL, Wilson L, Phiri A, Molyneux E, Zijlstra EE, Heyderman RS, Hart CA, Molyneux ME. (2008) Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *S. enterica* serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 46:963–969

Gordon MA (2008) *Salmonella* infections in immunocompromised adults*. J Infect* 56:413–422.

Gordon MA, Kankwatira AMK, Mwafulirwa G, Walsh AL, Hopkins MJ, Parry CM, Faragher EB, Zijlstra EE, Heyderman RS, Molyneux ME (2010) Invasive non-typhoid salmonellae establish systemic intracellular infection in HIV-infected adults: an emerging disease pathogenesis. *Clin Infect Dis* 50:953–962.

Gordon MA (2011) Invasive non-typhoidal *Salmonella* disease: epidemiology, pathogenesis and diagnosis *Current Opinion in Infectious* Diseases 24:484–489

Government of Kenya (2012) Meat Control Act vol 356 National Council for Law Reporting

Graham SM, Walsh AL, Molyneux EM, et al. (2000) Clinical presentation of non-typhoidal *Salmonella* bacteraemia in Malawian children. *Trans R Soc Trop Med Hyg;* 94:310–314

Graham SM, English M (2009) Non-typhoidal salmonellae: a management challenge for children with community-acquired invasive disease in tropical African countries*. Lancet;* 373:267–269.

Gray K, Wilson L, Phiri A, Corkill J, French N, Hart C (2006) Identification and characterization of ceftriaxone resistance and extended-spectrum beta lactamases in Malawian bacteraemic Enterobacteriaceae *J Ant Chem* 57:4, 661-665

Green SDR, Cheesbrough JS (1993) *Salmonella* bacteraemia among young children at a rural hospital in western Zaire. *Ann Trop Paediatr* 13:45–54.

Grimont P, Weill, F (2007) Antigenic Formulae of the Salmonella Serovars, (ninth ed.) Paris: WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur. Available at: http://www. pasteur.fr/sante/clre/cadrecnr/salmoms/WKLM_En.pdf.

Hall RM (2010) *Salmonella* genomic islands and antibiotic resistance in *Salmonella enterica*. Future Microbiol 5:1525–1538.

Hammarlöf D, Kroger C, Owen S, Canals R, Lacharme-Lora L, Wenner N, Schager A, Wells T, Henderson I, Wiglet P, Karsten H, Feasey N, Gordon M, Hinton J (2018) Role of a single noncoding nucleotide in the evolution of an epidemic African clade of *Salmonella Proc Natl Acad Sci USA* 115(11): E2614-E2623

Herenda D, Chambers PG, Ettriqui A, Seneviratna P, de Silva TJP (1994) Manual on meat inspection for developing countries. Rome: Food and Agriculture Organisation for the United Nations

Herrero-Fresno A, Wallrodt I, Leekitcharoenphon P, Olsen JE, Aarestrup FM, Hendriksen RS (2014) The role of the st313-td gene in virulence of *Salmonella* Typhimurium ST313. *PLoS One* 9:e84566.

Holmberg S, Wells J, Mitchell L (1984) Animal-to-man transmission of antimicrobial-resistant *Salmonella:* Investigates of U.S. Outbreaks, 1971-1983 Science 225(4664):833-835

Hopkins KL, Davies RH, Threlfall EJ (2005) Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int J Antimicrob Agents* 25:358 –373.

Huang J, Wang M, Ding H, Ye M, Hu F, Guo Q, Xu X, Wang M (2013) New Delhi metallo-β-lactamase-1 in carbapenem-resistant *Salmonella* strain, China. *Emerg Infect Dis* 19:2049–2051.

Hung C-C, Hung M-N, Hseuh P-R, Chang S-Y, Chen M-Y, Hsieh S-M, Sheng W-H, Sun H-Y, Lo Y-C, Hsiao C-F, Chang S-C (2007) Risk of recurrent non-typhoid *Salmonella* bacteremia in HIV-infected patients in the era of highly active anti-retroviral therapy and an increasing trend of fluoroquinolone resistance. *Clin Infect Dis* 45: e60 –67.

Ikwap K, Erume J, Okello Owiny D, Nasinyama GW, Melin L, Bengtsson B, Lundeheim N, Fellstrom C, Jacobson M (2014) *Salmonella* species in piglets and weaners from Uganda: Prevalence, antimicrobial resistance and herd-level risk factors *Prev Vet Med* 115(1-2) pp39-47

Irfan S, Khan E, Jabeen K, Bhawan P, Hopkins KL, Day M, Nasir A, Meunier D, Woodford N. (2015) Clinical isolates of *Salmonella enterica* serovar Agona producing NDM-1 metallo-β-lactamase: first report from Pakistan. *J Clin Microbiol* 53:346–348.

Iwu CJ, Iweriebor BC, Obi lc, Basson AK, Okoh AI (2016) Multidrug-Resistant Salmonella Isolates from Swine in the Eastern Cape Province, South Africa J Food Prod Jul;79(7):1234-9

Jones TF, Ingram LA, Cieslak PR, et al. (2008) Salmonellosis outcomes differ substantially by serotype. *J Infect Dis*; 198(1):109–114.

Josland SW (1954) The infective and immunogenic properties of *Salmonella* cholerasuis in weaner pigs *NZ Vet J* 2,41-46

Jure MA, Duprilot M, Musa HE, Lopez C, de Castillo MC, Weill FX, Arlet G, Decré D (2014) Emergence of KPC-2-producing *Salmonella enterica* serotype Schwarzengrund in Argentina. *Antimicrob Agents Chemother* 58:6335–6336.

Kagambega A, Lienemann T, Aulu L, Traore A, Barro N, Siitonen A, Haukka K (2013) Prevalence and characterization of Salmonella enterica from the feces of cattle, poultry, swine and hedgehogs in Burkino Faso and their comparison to human *Salmonella* isolates *BMC Microbiol* 13:253

Kankwatira AM, Mwafulirwa GAK, Gordon MA. (2004) Non-typhoidal *Salmonella* bacteremia: an under-recognized feature of AIDS in African adults. *Trop Doctor* 34:198 – 200.

Kariuki S. Gilks, C Corkhill J, Kimari J, Benea A, Waiyaki P, Hart C (1996) Multi-drug resistant non-typhi *Salmonella* in Kenya *J. Antimicrob Chemother.* 38, 425-434.

Kariuki, S (1997) Kenya: antibiotic resistance *The Lancet* Volume 349 S9 - S10

Kariuki S, Revathi G, Gayuka F, Yamo V, Muyodi J, Hart CA (2002) Lack of clonal relationship between non-typhi Salmonella strain types from humans and those isolated from animals living in close contact. *FEMS Immunol Med Microbiol* 33:165–171

Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Hart CA. (2006) Characterisation of community acquired non-typhoidal Salmonella from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. *BMC Microbiol* 6:101

Kariuki S, Revathi G, Kariuki N, Kiiru J, Mwituria J, Muyodi J, Githinji JW, Kagendo D, Munyalo A, Hart CA (2006) Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: zoonotic or anthroponotic transmission? *J Med Microbiol* 55:585–591.

Kariuki S, Gordon MA, Feasey N, Parry CM (2015) Antimicrobial resistance and management of invasive Salmonella disease *Vaccine* 19:33 Suppl 3: C21-9

Kebede A, Kemal J, Alemayehu H, Habte Mariam S (2016) Isolation, identification and antibiotic susceptibility testing of Salmonella from slaughtered bovines and ovines in Addis Ababa abbatori enterprise, Ethiopia; A cross-sectional study Int J Bacteriol, 3714785

Kingsley RA, Msefula CL, Thomson NR, Kariuki S, Holt KE, Gordon MA, Harris D, Clarke L, Whitehead S, Sangal V, Marsh K, Achtman M, Molyneux ME, Cormican M, Parkhill J, MacLennan CA, Heyderman RS, Dougan G (2009) Epidemic multiple drug resistant *Salmonella* Typhimurium causing invasive disease in sub-Saharan Africa have a distinct genotype. *Genome Res* 19:2279 –2287

Kikuvi G, Ombui J, Mitema E, Schwarz S (2007) Antimicrobial resistance in *Salmonella* serotypes isolated from slaughter animals in Kenya. *East Afr Med J*; 84(5): 233–239 (abstract only)

Kikuvi et al. (2010) Antimicrobial resistance in *Salmonella* isolates from pigs *J Infect Dev Ctries* 4(4):243-248.

Kikuvi G, Ombui J., Mitema E. (2010) Serotypes and antimicrobial resistance profiles of *Salmonella* isolates from pigs at slaughter in Kenya *J Inf Dis Ctries* 4(4) 243-248

Kishima, M, Uchida I, Namimatsu T, Osumi T, Takahashi S, Tanaka K, Aoki H, Matsuura K, and Yamamoto K (2008) Nationwide surveillance of *Salmonella* in the faeces of pigs in Japan. *Zoonoses Public Health* 55, 139–144.

Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, Faruque AS, Zaidi AK, Saha D, Alonso PL, Tamboura B, Sanogo D, Onwuchekwa U, Manna B, Ramamurthy T, Kanungo S, Ochieng JB, Omore R, Oundo JO, Hossain A, Das SK, Ahmed S, Qureshi S, Quadri F, Adegbola RA, Antonio M, Hossain MJ, Akinsola A, Mandomando I, Nhampossa T, Acácio S, Biswas K, O'Reilly CE, Mintz ED, Berkeley LY, Muhsen K, Sommerfelt H, Robins-Browne RM, Levine MM (2013) Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382:209 –222.

Kranker S, Alban L, Boes J, Dahl J, (2003) Longitudinal study of *Salmonella enterica* aerotype Typhimurium infection in three Danish farrow-to-finish swine herds *J Clin Microbiol* 41(6):228-8

Kulwichit W, Chatsuwan T, Unhasuta C, Pulsrikarn C, Bangtrakulnonth A, Chongthaleong A. (2007) Drug-resistant nontyphoidal *Salmonella* bacteremia, Thailand. *Emerg Infect Dis* 13:501–502.

Le Hello S, Harrois D, Bouchrif B, Sontag L, Elhani D, Guibert V, Zerouali K, Weill FX (2013) Highly drug-resistant *Salmonella enterica* serotype Kentucky ST198-X1: a microbiological study. *Lancet Infect Dis* 13:672–679

Lepage P, Bogaerts J, Van Goethem C, Ntahorutaba M, Nsengumuremyi F, Hitimana DG, Vandepitte J, Butzler J-P, Levy J. (1987) Community-acquired bacteremia in African children. *Lancet* i:1458 –1461.

Levings RS, Lightfoot D, Partridge SR, Hall RM, Djordjevic SP (2005) The genomic island SGI1, containing the multiple antibiotic resistance region of *Salmonella enterica* serovar Typhimurium DT104 or variants of it, is widely distributed in other *S. enterica* serovars. *J Bacteriol* 187:4401– 4409.

Lin LH, Tsai CY, Hung MH, et al. (2010) Rectal swab sampling followed by an enrichment culture-based real-time PCR assay to detect *Salmonella* enterocolitis in children. *Clin Microbiol Infect*

Lo Fo Wong DM, Dahl J, van der Wolf PJ, Wingstrand A, Leontides L, von Altrock A (2003) Recovery of *Salmonella enterica* from seropositive finishing pig herds Vet Microbiol 97:201-14

Mabey DCW, Brown A, Greenwood BM (1987) Plasmodium falciparum malaria and *Salmonella* infections in Gambian children. *J Infect Dis* 155: 1319 –1321

MacKenzie G, Ceesay SJ, Hill PC, Walther M, Bojang KA, Satoguina J, Enwere G, D'Alessandro U, Saha D, Ikumapayi UN, O'Dempsey T, Mabey DC, Corrah T, Conway DJ, Adegbola RA, Greenwood BM (2010) A decline in the incidence of invasive non-typhoidal

Salmonella infection in the Gambia temporally associated with a decline in malaria infection. *PLoS One* 11;5(5): e10568

MacLennan C, Fieschi C, Lammas DA, Picard C, Dorman SE, Sanal O, MacLennan JM, Holland SM, Ottenhoff TH, Casanova JL, Kumara (2004) Interleukin (IL)-12 and IL-23 are key cytokines for immunity against *Salmonella* in humans *J Infect Dis* 190(10):1755-7

MacLennan CA, Gondwe EN, Msefula CL, Kingsley RA, Thomson NR, White SA, Goodall M, Pickard DJ, Graham SM, Dougan G, Hart CA, Molyneux ME, Drayson MT (2008) The neglected role of antibody in protection against bacteremia caused by non-typhoidal strains of *Salmonella* in African children*. J Clin Invest* 118:1553–1562.

MacLennan CA, Gilchrist JJ, Gordon MA, Cunningham AF, Cobbold M, Goodall M, Kingsley RA, van Oosterhout JJ, Msefula CL, Mandala WL, Leyton DL, Marshall JL, Gondwe EN, Bobat S, López Macías C, Doffinger R, Henderson IR, Zijlstra EE, Dougan G, Drayson MT,

MacLennan IC, Molyneux ME (2010) Dysregulated humoral immunity to non-typhoidal *Salmonella* in HIV-infected African adults. *Science* 328: 508 –512.

MacLennan C, Levine M (2013) Invasive non-typhoidal Salmonella disease in Africa: current status *Expert Rev Anti Infect Ther* 11(5):443-6

MacLennan CA, Martin LB, Micoli F (2014) Vaccines against invasive *Salmonella* disease: current status and future directions. *Hum Vaccin Immunother* 10:1478–93.

MacLennan CA, Saul A. (2014) Vaccines against poverty. *Proc Natl Acad Sci USA* 111:12307–12312

Majowicz SE, Musto J, Scallan E, Angulo FJ, O'Brein SJ, Jones TF (2010) The global burden of non-typhoidal *Salmonella* gastroenteritis. *Clin Infect Dis*.50:882–9

Maka L, Mackiw E, Sciezynska H, Pawlowska K, Popowska M (2014) Antimicrobial susceptibility of *Salmonella* strains isolated from retail meat products in Poland between 2008 and 2012 Food Control 36(1) 199-204

Makoka M, Miller W, Hoffman I, Cholera R, Gilligan P, Kamwendo D, Malunga G, Joaki G, Martinson F, Hosseinipour M (2012) Bacterial infections in Lilongwe, Malawi; aetiology and antibiotic resistance *BMC Infectious Disease* 12:67

Malorny B, Paccassoni E, Fach P, Bunge C, Martin A, Helmuth R (2004) Diagnostic real-time PCR for detection of Salmonella in food *Appl Environ Microbiol* 70(12):7046-7052

Mann I (1984) Guidelines on small slaughterhouses and meat hygiene in developing countries. Edited by Koulikovski A, Mayas Z Geneva: World Health Organisation

Marshall WF, Blair JE (1999) The cephalosporins. *Mayo Clin Proc* 74: 187–195.

Mathole MA, Muchadeyi FC, Mdladla K, Malatji DP, Dzomba EF, Madoroba E (2017) Presence, distribution, serotypes and antimicrobial resistance profiles of *Salmonella* among pigs, chickens and goats in South Africa *J Food Cont* 72B; 219-224

Martin LB (2012) Vaccines for typhoid fever and other salmonelloses. *Curr Opin Infect Dis*; 25:489–99.

Martinez-Martinez L, Pascual A, Jacoby GA. (1998) Quinolone resistance from a transferable plasmid. *Lancet* 351:797–799

McClelland M, Sanderson K, Spieth, J, Clifton, S, Latreille, P, Courtney, L, Wilson,R (2001). Complete genome sequence of *Salmonella enterica* serovar Typhimurium LT2. *Nature*, *413*, 852.

McFarland (1907) Nephelometer: An instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *J Amer Med Assoc* 14:1176

McKinley GA, Fagerberg DJ Quarles, B.A. George, D.E. Wagner, and L.D. Rollins (1980) Incidence of salmonellae in fecal samples of production swine and swine at slaughter plants in the United States in 1978. *Appl. Environ. Microbiol*. 40, 562–566.

Milledge J, Calis JC, Graham SM, et al. (2005) Aetiology of neonatal sepsis in Blantyre, Malawi: 1996–2001. *Ann Trop Paediatr;* 25:101–110.

Miriagou V, Tassios PT, Legakis NJ, Tzouvelekis LS (2004) Expanded spectrum cephalosporin resistance in non-typhoid *Salmonella. Int J Antimicrob Agents* 23:547–555

Mishu B, Koehler J, Lee l, Rodrigue D, Brenner F, Blake P, Tauxe R (1994) *J Infect Dis* 169(3):547-52

Mitema E, Kikuvi G (2004) Surveillance of the overall use of antimicrobial drugs in humans over a 5 year period (1997-2001) in Kenya *Journal of Antimicrobial Chemotherapy*

Morgan IR, Krautil FL, Craven JA (1987) Effect of time in lairage on caecal and carcass *Salmonella* contamination of slaughter pigs. *Epidemiol Infect* 98:323-30

Morpeth SC, Ramadhani HO, Crump JA (2009) Invasive non-Typhi *Salmonella* disease in Africa. *Clin Infect Dis* 49:606 –611

Mtove G, Amos B, Nadjm B, Hendriksen IC, Dondorp AM, Mwambuli A, Kim DR, Ochiai RL, Clemens JD, von Seidlein L, Reyburn H, Deen J (2011) Decreasing incidence of severe malaria and community acquired bacteraemia among hospitalized children in Muheza, northeastern Tanzania, 2006-2010. *Malar J* 10:320.

Muischa P, Cornick J, Bar-Zeev N, French N, Massesa C, Denis B, Kennedy N, Mallewa J, Gordon M, Msefula C, Heyderman R, Everett D, Feasey N (2017) Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998-2016): a surveillance study *Lancet* 17(10) 1042-1052

Nadjm B, Amos B, Mtove G, et al. (2010) WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study. *BMJ* 2010; 340:c1350

Nastasi A, Mammina C, Villafrate MR, Massenti MF, Scarlata G et al. (1988) Multiple typing of strains of *Salmonella enterica* subsp. *bongori* ser. 48:Z35:- isolated in southern Italy. *Ann Inst Pasteur Microbiol.* 139:605–612.

Nollet N, Maes d, Duchateau L, Hautekiet V, Houf K, Van Hoof J, De Zuttera L, De Kruif A, Geers R (2005) Discrepancies between the isolation of *Salmonella* from mesenteric lymph nodes and the results of serological screening in slaughter pigs *Vet Res* 36: 545-55

Nyirenda TS, Gilchrist JJ, Feasey NA, Glennie SJ, Bar-Zeev N, Gordon MA, MacLennan CA, Mandala WL, Heyderman RS (2014) Sequential acquisition of T cells and antibodies to non-typhoidal *Salmonella* in Malawian children. *J Infect Dis* 210:56 –64.

O'Brien JDP (1966) An outbreak of *Salmonella* cholerasuis infection in a large pig fattening enterprise in *Lancashire Vet Rec* 79; 558-561

O'Dempsey TJ, McArdle TF, Lloyd-Evans N, Baldeh I, Laurence BE, Secka O, Greenwood BM (1994) Importance of enteric bacteria as a cause of pneumonia, meningitis and septicemia among children in a rural community in The Gambia, West Africa. *Pediatr Infect Dis J* 13: 122–128.

Okoro CK, Kingsley RA, Quail MA, Kankwatira AM, Feasey NA, Parkhill J, Dougan G, Gordon MA (2012) High-resolution single nucleotide polymorphism analysis distinguishes recrudescence and reinfection in recurrent invasive non-typhoidal *Salmonella* Typhimurium disease. *Clin Infect Dis* 54:955–963.

Okoro CK, Kingsley RA, Connor TR, Harris SR, Parry CM, AlMashhadani MN, Kariuki S, Msefula CL, Gordon MA, de Pinna E, Wain J, Heyderman RS, Obaro S, Alonso PL, Mandomando I, MacLennan CA, Tapia MD, Levine MM, Tennant SM, Parkhill J, Dougan G (2012) Intracontinental spread of human invasive *Salmonella* Typhimurium pathovariants in sub-Saharan Africa. *Nat Genet* 44:1215–1221.

Okoro C, Barquist L, Connor T, Harris S, Clare S, Stevens M, Arends M, Hale C, Kane L, Pickard D, Hill J, Harcourt K, Parkhill J, Dougan G, Kingsley R (2015) Singatures of adaptation in human invasive Salmonella Typhimurium ST313 populations from sub-Saharan Africa PLOS Neglected Tropical Diseases 9(6) e0003848

Paglietti B, Falchi G, Mason P, Chitsatso O, Nair S, Gwanzura L, Uzzau S, Cappuccinelli P, Wain J, Rubino S (2013) Diversity among human non-typhoidal *Salmonellae* isolates from Zimbabwe. *Trans R Soc Trop Med Hyg* 107:487–492

Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, Griffin PM (2013) Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998-2008. *Emerg Infect Dis* 19:407–415.

Parry CM (2003) Antimicrobial drug resistance in *Salmonella* enterica. *Curr Opin Infect Dis* 16:467–472.

Parry CM, Thomas S, Aspinall EJ, Cooke RP, Rogerson SJ, Harries AD, Beeching NJ (2013) A retrospective study of secondary bacteraemia in hospitalised adults with community acquired non-typhoidal *Salmonella* gastroenteritis. *BMC Infect Dis* 13:107.

Parsons BN, Humphrey S, Salisbury AM, Mikoleit J, Hinton JC, Gordon MA, Wigley P (2013) Invasive non-typhoidal *Salmonella* Typhimurium ST313 are not host-restricted and have an invasive phenotype in experimentally infected chickens. *PLoS Negl Trop Dis* 7:e2487

Paterson DL, Bonomo RA. (2005) Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 18:657–686.

Peters RPH, Zijlstra EE, Schijffelen MJ, et al. (2004) A prospective study of bloodstream infections as a cause of fever in Malawi – clinical predictors and implications for management. *Trop Med Int Health* 9:928– 934.

Philippon A, Arlet G, Jacoby GA (2002) Plasmid-determined AmpCtype beta-lactamases. *Antimicrob Agents Chemother* 46:1–11.

Pires SM, DE Kneg l, Hald T (2011) Estimation of the relative contribution of different food and animal sources to human *Salmonella* infections in the European Union Available from: http://www.efsa.europa.eu/en/supporting/doc/184e.pdf. Accessed 21 August 2017

Poppe, C, Smart, N, Khakhria R, Johnson W, Spika J, Prescott, J (1998). *Salmonella* typhimurium DT104: a virulent and drug-resistant pathogen. *The Canadian Veterinary Journal*, *39*(9), 559–565.

Pornruangwong S, Hendriksen RS, Pulsrikarn C, Bangstrakulnonth A, Mikoleit M, Davies RH, Aarestrup FM, Garcia-Migura L (2011) Epidemiological investigation of *Salmonella enterica* serovar Kedougou in Thailand. *Foodborne Pathog Dis* 8:203–211

Porwollik S, Boyd EF, Choy et al. (2004) Characterisation of *Salmonella enterica* subspecies 1 genovars by use of microarrays *J Bacteriol 196*:5883-98

Poudel S, Shrestha S, Pradham A, Sapkota B, Mahato M (2014) Antimicrobial susceptibility pattern of salmonella enterica species in blood culture isolates *Clin Micro* 3:141

Preziosi MJ, Kandel SM, Guiney DG, Browne SH (2012) Microbiological analysis of non-typhoidal *Salmonella* strains causing distinct syndromes of bacteremia or enteritis in HIV/AIDS patients in San Diego, California. *J Clin Microbiol* 50:3598 –3603.

Profeta S, Forrester C, Eng Liu RHR, Johnson E, Palinkas R, Smith SM (1985) *Salmonella* infections in patients with acquired immunodeficiency syndrome. *Arch Intern Med* 145:670 –672.

Qamar FN, Azmatullah A, Bhutta ZA. (2015) Challenges in measuring complications and death due to invasive *Salmonella* infections. *Vaccine*; 33(suppl3):C16–20.

Raffatellu M, Santos RL, Verhoeven DE, George MD, Wilson RP, Winter SE, Godinez I, Sankaran S, Paixao TA, Gordon MA, Kolls JK, Dandekar S, Baumler AJ. (2008) Simian immunodeficiency virus induced mucosal interleukin-17 deficiency promotes Salmonella dissemination from the gut. *Nat Med* 14:421–428

Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, Savard P, Humphries RM, Kallen AJ, Limbago BM (2013) New Delhi metallo-β-lactamase-producing Enterobacteriaceae, United States. *Emerg Infect Dis* 19:870–878.

Reddy EA, Shaw AV, Crump JA (2010) Community acquired bloodstream infections in Africa: a systematic review and meta-analysis. *Lancet Infect Dis* 10:417–432.

Rodríguez E, Bautista A, Barrero L (2014) First report of a *Salmonella enterica* serovar Typhimurium isolate with carbapenemase (KPC-2) in Colombia. *Antimicrob Agents Chemother* 58:1263–1264.

Rutherford ME, Mulholland K, Hill PC (2010) How access to health care relates to under-five mortality in sub-Saharan Africa: systematic review. *Trop Med Int Health* 15:508–19.

Schreiber F, Lynn DJ, Houston A, Peters J, Mwafulirwa G, Finlay BB, Brinkman FS, Hancock RE, Heyderman RS, Dougan G, Gordon MA (2011) The human transcriptome during non-typhoid *Salmonella* and HIV coinfection reveals attenuated NFkappaB-mediated inflammation and persistent cell cycle disruption. *J Infect Dis* 204:1237–1245.

Scott JA, Berkley JA, Mwangi I, Ochola L, Uyoga S, Macharia A, Ndila C, Lowe BS, Mwarumba S, Bauni E, Marsh K, Williams T (2011) Relation between falciparum malaria and bacteraemia in Kenyan children: a population-based, case-control study and a longitudinal study. *Lancet* 378:1316 – 1323

Sekyere J, Adu F (2015) Prevalence of multidrug resistance among Salmonella enterica serovar Typhimurium isolated from pig faeces in Ashanti region, Ghana *Int Jour Antib* Article ID 898790 4 pages

Sigauque B, Roca A, Mandomando I, Morais L, Quinto L, Sacarlal J, Macete E, Nhamposa T, Machevo S, Aide P, Bassat Q, Bardaji A, Nhalungo D, Soriano-Gabarro M, Flannery B, Menendez C, Levine MM, Alonso PL (2009) Community-acquired bacteremia among children admitted to a rural hospital in Mozambique. *Pediatr Infect Dis J* 28:108 –113.

Siggins MK, O'Shaughnessy CM, Pravin J, Cunningham AF, Henderson IR, Drayson MT, MacLennan CA (2014) Differential timing of antibody-mediated phagocytosis and cell-free killing of invasive African *Salmonella* allows immune evasion. *Eur J Immunol* 44:1093–1098.

Sirichote P, Hasman H, Pulsrikarn C, Schonheyder HC, Samulioniene J, Pornruangmong S, Bangtrakulnonth A, Aarestrup FM, Hendriksen RS (2010) Molecular characterization of extended-spectrum cephalosporinase-producing *Salmonella enterica* serovar Choleraesuis isolates from patients in Thailand and Denmark. *J Clin Microbiol* 48:883–888.

Sirichote P, Bangtrakulnonth A, Tianmanee K, Unahalekhaka A, Oulai A, Chittaphithakchai P, Kheowrod W, Hendriksen RS (2010) Serotypes and antimicrobial resistance of *Salmonella enterica spp* in central Thailand, 2001-2006. *Southeast Asian J Trop Med Public Health* 41:1405– 1415.

Sommet J, Missud F, Holvoet L, Ithier G, Lorrot M, Benkerrou M, Faye A (2013) Morbidity among child travellers with sickle-cell disease visiting tropical areas: an observational study in a French tertiary care centre. *Arch Dis Child* 98:533–536.

Strahilevitz J, Jacoby GA, Hooper DC, Robicsek A (2009) Plasmid-mediated quinolone resistance: a multifaceted threat. *Clin Microbiol Rev* 22:664 –689.

Tabu C, Breiman RF, Ochieng B, Aura B, Cosmas L, Audi A, Olack B, Bigogo G, Ongus JR, Fields P, Mintz E, Burton D, Oundo J, Feikin DR (2012) Differing burden and epidemiology of non-Typhi *Salmonella* bacteremia in rural and urban Kenya, 2006-2009. *PLoS One* 7:e31237.

Talbert AW, Mwaniki M, Mwarumba S, et al. (2010) Invasive bacterial infections in neonates and young infants born outside hospital admitted to a rural hospital in Kenya. *Pediatr Infect Dis J* 29:945–949.

Tennant SM, Diallo S, Levy H, et al. (2010) Identification by PCR of non-typhoidal *Salmonella enterica* serovars associated with invasive infections among febrile patients in Mali. *PLoS Negl Trop Dis* 4:e621.

Thomas L, de Glanville WA, Cook EA. Fèvre EM (2013) The spatial ecology of free-ranging domestic pigs (Sus scrofa) in western Kenya *BMC Veterinary Research* 9:46

Threlfall EJ. (2000) Epidemic *Salmonella* Typhimurium DT 104: a truly international multi-resistant clone. *J Antimicrob Chemother* 46:7–10.

Threlfall EJ (2002) Antimicrobial drug resistance in *Salmonella*: problems and perspectives in food- and water-borne infections. *FEMS Microbiol Rev* 26:141–148.

Tiong V, Thong KL, Yusof MY, Hanifah YA, Sam JI, Hassan H (2010) Macrorestriction analysis and antimicrobial susceptibility profiling of *Salmonella enterica* at a University Teaching Hospital, Kuala Lumpur. *Jpn J Infect Dis* 63:317–322.

Trebicka E, Shanmugam NK, Mikhailova A, Alter G, Cherayil BJ (2014) Effect of human immunodeficiency virus infection on plasma bactericidal activity against *Salmonella enterica* serovar Typhimurium. *Clin Vaccine Immunol* 21:1437–1442.

Turner AK, Nair S, Wain J (2006) The acquisition of full fluoroquinolone resistance in *Salmonella* Typhi by accumulation of point mutations in the topoisomerase targets. *J Antimicrob Chemother* 58:733–740.

Uche I, MacLennan C, Saul A (2017) A systematic review of the incidence, risk factors and case fatality rates of invasive nontyphoidal Salmonella (Ints) disease in Africa (1966 to 2014) PLoS Negl Trop Dis 11(1)

Uzzau S, Brown DJ, Wallis T, Rubino S, Leori G, Bernard S, Casadesus J, Platt DJ, Olsen JE (2000). Host adapted serotypes of Salmonella enterica. *Epidemiol. Infect.* 125:229–255

van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, Español T, Fischer A, Kurenko-Deptuch M, Mouy R, Petropoulou T, Roesler J, Seger R, Stasia MJ, Valerius NH, Weening RS, Wolach B, Roos D, Kuijpers TW (2009) Chronic granulomatous disease: the European experience. *PLoS One* 4

Varma JK, Molbak K, Barrett TJ, Beebe JL, Jones TF, Rabatsky-Ehr T, Smith KE, Vugia DJ, Chang H-GH, Angulo FJ (2005) Antimicrobial resistant nontyphoidal *Salmonella* is associated with excess bloodstream infections and hospitalizations. *J Infect Dis* 191:554 –561.

Vo AT, van Duijkeren E, Gaastra W, Fluit AC (2010) Antimicrobial resistance, class 1 integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. *PLoS One* 5:e9440.

Vugia DJ, Samuel M, Farley MM, Marcus R, Shiferaw B, Shallow S, Smith K, Angulo FJ, for the Emerging Infections Program FoodNet Working Group (2004) Invasive *Salmonella* infections in the United States, FoodNet, 1996-1999: incidence, serotype distribution, and outcome. *Clin Infect Dis* 38(Suppl 3):S149 –S156.

Wales AD, Cook AJ, Davies RH. 2011. Producing *Salmonella*-free pigs: a review focusing on interventions at weaning. Vet Rec 168:267–76

Wannaprasat W, Padungtod P, Chuanchuen R (2011) Class 1 integrons and virulence genes in *Salmonella enterica* isolates from pork and humans. *Int J Antimicrob Agents* 37:457–461.

Watera C, Nakiyingi J, Miiro G, Muwonge R, Whitworth JA, Gilks CF, French N (2004) 23-Valent pneumococcal polysaccharide vaccine in HIV infected Ugandan adults: 6-year follow-up of a clinical trial cohort. *AIDS* 18:1210 –1213

Wigley P (2017) Salmonella enterica serovar Gallinarum: addressing fundamental questions in bacteriology sixty years on from the 9R vaccine, Avian Pathology, 46:2, 119-124

Wilhelm B, Young I, Cahill S, Nakagawa R, Desmarchelier P, Rajic A (2017) Rapid systematic review and meta-analysis of evidence for effectiveness of primary production interventions to control Salmonella in beef and pork *Prev Vet Med* 147:213-225

WHO Factsheet (Available online at WHO website; http://www.who.int/mediacentre/factsheets/fs139/en/) Last Accessed 19.4.17

Wood RL, Pospischil A, Rose R (1989) Distribution of persistent Salmonella typhimurium infection in internal organs of swine *Am J Vet Res* 50: 1015-21

Wright JG, Tengelsen LA, Smith KE, Bender JB, Frank RK, Grendon JH, Rice DH, Thiessen AM, Gilbertson CJ, Sivapalasingam S, Barrett TJ, Besser TE, Hancock DD, Angulo FJ (2005) Multidrug-resistant *Salmonella* Typhimurium in four animal facilities *Emerg Infect Dis* 11(8):1235-41

Yang J, Barrila J, Roland KL, Kilbourne J, Ott CM, Forsyth RJ, Nickerson CA (2015) Characterization of the invasive, multidrug resistant non-typhoidal *Salmonella* strain D23580 in a murine model of infection. *PLoS Negl Trop Dis*

Zhang S, Kingsley R, Santos R, Andrews-Polymenis H, Raffatellu M, Figueiredo J, Bäumler A (2003) Molecular Pathogenesis of *Salmonella enterica* Serotype Typhimurium-Induced Diarrhea . *Infection and Immunity*, *71*(1), 1–12.

Appendix

Contents of Appendix

Table 1 shows the complete results of all positive isolates of NTS currently returned from whole genome sequencing. The majority of this isolates are 'unique' isolates, i.e. they are single isolates of NTS which originate from pigs and hold differing antimicrobial susceptibility phenotypes, serovars and sequence types from the other all other isolates determined, even from other isolates found within the same pig. Within this table there are also 11 duplicate isolates included. These duplicates are two isolates which originate from the same pig, found in the same location (either mesenteric lymph node or faecal sample

pig) as another and share the same serovar, sequence type and antimicrobial resistance profile; therefore for the purpose of this study they are considered identical. One of each duplicate isolate of each pair has been highlighted in bold in the table for ease of identification.

Map data shows the locations of slaughterslabs and sample collection In Busia (Map I), Chikwawa Valley (Map III). Map II and Map IV show the location of pigs reared from which samples were collected in Busia and Nairobi. The location of rearing of pigs in Malawi was not able to be determined during sample collection.

KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
8	315	FD01844656	Busia	6mo	mixed, pink and black		Mujuru	Mundika	Motorbike		MLNHAC	y	y	y	Guildford	11	Susceptible	S	S	S	S	S		S	S	S	S	S	S
8	321	FD01543576	Busia	6mo	mixed, pink and black	mn	Mujuru	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Guildford	4288	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S
17	316	FD01543537	Busia	8mo	Unknown	f	Khuyala, Matayso	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Stanleyville	4291	Resistant to ampicillin and tetracycline	R	S	S	R	S	S	S	S	S	S	S	I
22	210	FD01543573	Busia	7mo	white	m	Khungungu	Mundika	Motorbike	Unknown	FBGA	n	y	y	Guildford	4288	Susceptible	S	S	S	S	S	S	S	S	S	S	S	
25	322	FD01844648	Busia	12mo	white	f	Khungungu	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Newport	46	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
36	213	FD01543534	Busia	3mo	black	f	Nasira	Mundika	Motorbike	Unknown	FBGA	y	y	y	Heidelberg	4290	Susceptible	S	S	S	S	S	S	S	S	S		S	
37	211	FD01543565	Busia	3mo	white	f	Nasira	Mundika	Motorbike	Unknown	FHAC	y	y	y	Heidelberg	4290	Susceptible	S	S	S	S	S	S	S	S	S	S	S	
52	313	FD01844664	Busia	8mo	black and white	m	Mudokomi, Mundika	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Newport	46	Susceptible	s	s	s	s	s	s	s	s	s	s	s	s
61	214	FD01543509	Busia	9mo	white	mn	Siriba, Matayos	Bumala	Motorbike	No	FBGA	y	y	y	Newport	46	Susceptible	S	S	S	S	S	S	S	S	I	S	S	S
61	306	FD01844585	Busia	9mo	white	mn	Siriba, Matayos	Bumala	Motorbike	No	MLNHAC	y	y	y	Newport	46	Susceptible	S	S	S	S	S		S	S	S	S	S	S
61	**311**	**FD01543520**	**Busia**	**9mo**	**white**	**mn**	**Siriba, Matayos**	**Bumala**	**Motorbike**	**No**	**MLNBGA**	**y**	**y**	**y**	**Newport**	**46**	**Susceptible**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**I**	**S**	**S**	**S**
62	317	FD01543529	Busia	12mo	white	f	Murambia	Bumala	Motorbike	Unknown	MLNHAC	y	y	y	Newport	46	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S

KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
63	298	FD01543516	Busia	12mo	white	m	Bumala	Bumala	Motorbike	Unknown	MLNHAC	y	y	y	Newport	46	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
63	**318**	**FD01543553**	**Busia**	**12mo**	**white**	**m**	**Bumala**	**Bumala**	**Motorbike**	**Unknown**	**MLNBGA**	**y**	**y**	**y**	**Newport**	**46**	**Susceptible**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
72	308	FD01844672	Busia	5-6mo	black	unknown	Mundika	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Stanleyville	1986	MDR	R	S	R	R	R		S	S	R	R	R	I
72	319	FD01543545	Busia	5-6mo	black	unknown	Mundika	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Stanleyville	912	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
75	299	FD01543508	Busia	14mo	white	f	Siteko	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Guildford	4288	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S
76	219	FD01543589	Busia	6mo	white	f	Kubringala, Mundika	Mundika	Motorbike	Unknown	FHAC	n	y	y	Guildford	4288	Susceptible	S	S	I	S	S	S	S	S	S	S	S	
81	301	FD01844601	Busia	6mo	white	m	Nwamonge, Bumala	Bumala	Motorbike	Unknown	MLNHAC	y	y	y	Heidelberg	15	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
83	303	FD01844593	Busia	9mo	black	f	Eburiang, Sikulu	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Fulica	1063	Susceptible	s	s	i	s	s	s	s	s	s	s	s	s
83	**304**	**FD01543557**	**Busia**	**9mo**	**black**	**f**	**Eburiang, Sikulu**	**Mundika**	**Motorbike**	**Unknown**	**MLNHAC**	**Y**	**y**	**Y**	**Fulica**	**1063**	**Susceptible**	**s**	**s**	**i**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**
85	290	FD01543533	Busia	12mo	black	m	Sirisia, Bugengi	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
85	291	FD01543524	Busia	12mo	black	m	Sirisia, Bugengi	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	No serovar	ND	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S

KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughtersiab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
86	293	FD01543588	Busia	10mo	black and white	m	Mudokomi, Mundika	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	No Salmonella identified	ND	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
86	305	FD01543549	Busia	10mo	black and white	m	Mudokomi, Mundika	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
97	288	FD01543564	Busia	6mo	white	f	Mabunge, Nambale	Nambale	Motorbike	Unknown	LNHAC	Y	y	Y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
113	295	FD01543541	Busia	7mo	unknown	m	Mohuyu, Kisoko	Mugatsi	Motorbike	Treated, unsure what or when	MLNBGA	y	y	y	Offa	-319	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
116	221	FD01543542	Busia	18mo	white	f	Kagina, Bujumba	Bumala	Motorbike	Treated 1 month ago, unknown drug	FBGA	y	y	y	Heidelberg	4292	Susceptible	S	S	I	S	S	S	S	S	S	S	S	
116	279	FD01543563	Busia	18mo	white	f	Kagina, Bujumba	Bumala	Motorbike	Treated 1 month ago, unknown drug	MLNBGA	y	y	y	Heidelberg	4292	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
121	286	FD01543556	Busia	Unknown	unknown	unknown	Unknown	Unknown	Motorbike	Unknown	MLNHAC	y	y	y	Enteritidis	11	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S
140	**270**	**FD01543522**	**Busia**	**9mo**	**white**	**m**	**Buringala, Mundika**	**Mundika**	**Motorbike**	**Unknown**	**MLNBGA**	**y**	**y**	**y**	**Newport**	**46**	**Susceptible**	**S**	**S**	**I**	**S**	**S**	**S**	**S**	**S**	**I**	**S**	**S**	**S**
140	277	FD01543579	Busia	9mo	white	m	Buringala, Mundika	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Newport	46	Susceptible	S	S	I	S	S	S	S	I	I	S	S	S
144	276	FD01543555	Busia	9mo	black and white	f	Buyende, Mundika	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Uganda	684	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S

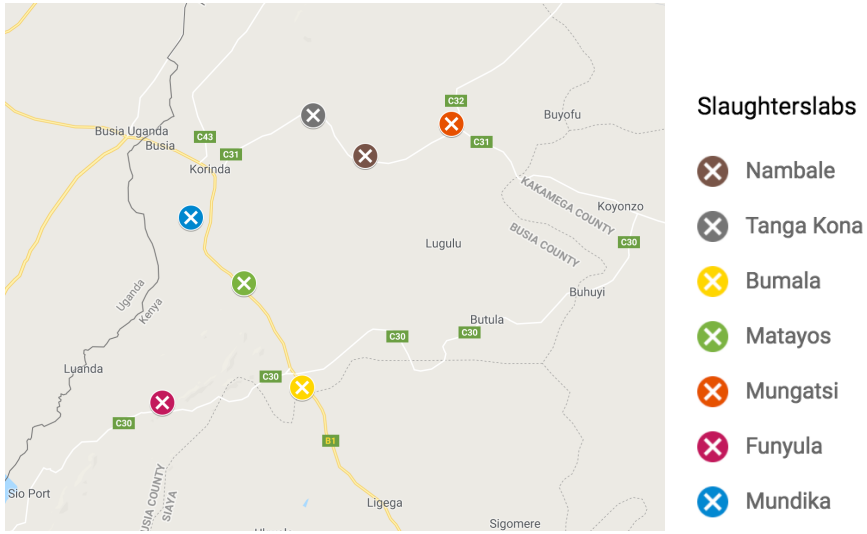
KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
144	**287**	**FD01543548**	**Busia**	**9mo**	**black and white**	**f**	**Buyende, Mundika**	**Mundika**	**Motorbike**	**Unknown**	**MLNBGA**	**y**	**y**	**Y**	**Uganda**	**684**	**Susceptible**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**I**	**S**	**S**	**S**
146	**269**	**FD01543531**	**Busia**	**5mo**	**black and white**	**m**	**Mundika**	**Mundika**	**motorbike**	**Unknown**	**FBGA**	**y**	**y**	**y**	**Uganda**	**684**	**Susceptible**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**I**	**S**	**S**	**S**
146	273	FD01543539	Busia	5mo	black and white	m	Mundika	Mundika	motorbike	Unknown	FHAC	y	y	y	Uganda	684	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S
155	167	FD01844631	Busia	6mo	unknown	m	Bugenyi	Mundika	Motorbike	Unknown	FHAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	I	I	S	S	S
155	168	FD01844639	Busia	6mo	unknown	m	Bugenyi	Mundika	Motorbike	Unknown	FBGA	y	y	Y	Fulica	1063	MDR	R	R	S	S	S		I	R	R	R	S	R
155	241	FD01543532	Busia	6mo	unknown	m	Bugenyi	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Heidelberg	15	Resistant to chloramphenicol and cefotaxime	S	R	I	S	S	S	S	S	S	R	S	I
179	243	FD01543540	Busia	8mo	unknown	f	Bumala	Bumala	Motorbike	Unknown	MLNBGA	y	y	y	Heidelberg	15	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
189	257	FD01543527	Busia	12mo	unknown	f	Sikulu	Mundika	Motorbike	Unknown	MLNHAC	y	y	y	Aberdeen	4038	Susceptible	S	S	R	S	S	S	S	S	I	S	S	S
202	200	FD01543581	Busia	24mo	unknown	unknown	Funyula	Funyula	Motorbike	Unknown	FHAC	y	y	y	Uganda	684	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S
212	233	FD01543582	Busia	6mo	white	f	Lwanikha,	Nambale	Motorbike	Unknown	MLNBGA	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	
253	133	FD01844663	Busia	12mo	black and white	f	Busibwabo	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Bovismorbificans	142	Resistant to streptomycin and tetracycline	S	S	i	R	S	S	S	S	S	S	S	S
255	124	FD01543536	Busia	6mo	black	f	Sirisia, Bugengi	Mundika	Motorbike	Unknown	MLNBGA	y	y	y	Tilene	912	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S

KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
255	**127**	**FD01844584**	**Busia**	**6mo**	**black**	**f**	**Sirisia, Bugengi**	**Mundika**	**Motorbike**	**Unknown**	**MLNHAC**	**Y**	**y**	**Y**	**Tilene**	**912**	**Susceptible**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**	**s**
257	157	FD01844671	Busia	12mo	unknown	m	Logingo, Matayos	Bumala	Motorbike	Unknown	FBGA	y	y	y	Newport	166	Susceptible	S	S	I	S	S		S	S	I	S	S	S
26	15	FD01543546	Nairobi	unknown	Landrace cross	unknown	Dandora	Ndumboini slaughterhouse	Pickup truck	Unknown	MLNHAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S
31	340	FD01844616	Nairobi	unknown	Landrace cross	unknown	Ruiru	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y	y	y	Muenchen	82	Susceptible	S	S	I	S	S		S	S	S	S	S	S
35	349	FD01543561	Nairobi	unknown	Landrace cross	unknown	Githunguri	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y	y	y	Muenchen	82	Susceptible	S	S	I	S	S		S	S	I	S	S	S
35	**379**	**FD01543499**	**Nairobi**	**unknown**	**Landrace cross**	**unknown**	**Githunguri**	**Ndumboini slaughterhouse**	**Pickup truck**	**Unknown**	**FHAC**	**y**	**y**	**y**	**Muenchen**	**82**	**Susceptible**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
66	352	FD01844613	Nairobi	unknown	Landrace cross	m	Githiga	Ndumboini slaughterhouse	Pickup truck	Unknown	MLNHAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S
70	353	FD01543506	Nairobi	unknown	Landrace cross	m	Unknown	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y	y	Y	Typhimurium	19	No results												
71	360	FD01844653	Nairobi	unknown	Landrace cross	f	Unknown	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y	y	y	Typhimurium	19	Resistant to streptomycin and tetracycline	S	S	R	R	S	S	S	S	S	S	S	S
71	**361**	**FD01844673**	**Nairobi**	**unknown**	**Landrace cross**	**f**	**Unknown**	**Ndumboini slaughterhouse**	**Pickup truck**	**Unknown**	**FHAC**	**y**	**y**	**y**	**Typhimurium**	**19**	**Resistant to streptomycin and tetracycline**	**S**	**S**	**R**	**R**	**S**		**S**	**S**	**S**	**S**	**S**	**S**
94	323	FD01844661	Nairobi	unknown	Landrace cross	unknown	Banana	Ndumboini slaughterhouse	Pickup truck	Unknown	MLNBGA	y	y	y	Kiambu	309	Susceptible	S	S	I	S	S		S	S	S	S	I	S
112	73	FD01844594	Nairobi	unknown	Landrace cross	m	Kenyariri	Ndumboini slaughterhouse	Pickup truck	Unknown	MLNHAC	y	y	y	Typhimurium	19	Resistant to streptomycin and tetracyline	S	S	R	R	S	S	S	S	S	S	S	S

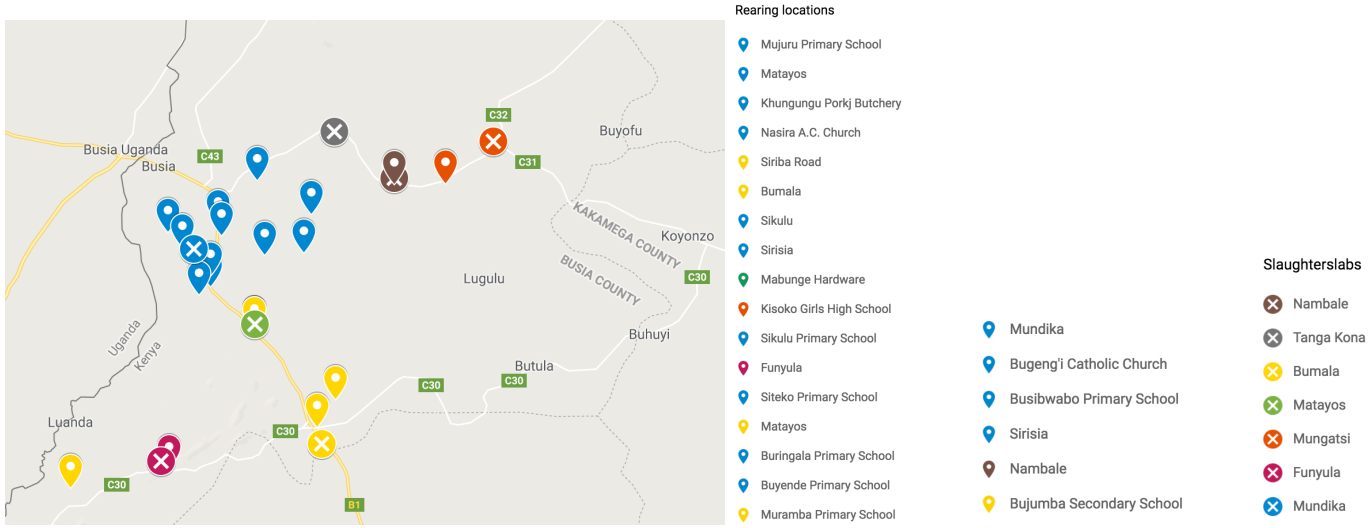
KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
112	**342**	**FD01844614**	**Nairobi**	**unknown**	**Landrace cross**	**m**	**Kenyarii**	**Ndumboini slaughterhouse**	**Pickup truck**	**Unknown**	**MLN BGA**	**y**	**y**	**y**	**Typhimurium**	**19**	**Resistant to streptomycin and tetracycline**	**S**	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
131	100/403	FD01844623	Nairobi	unknown	Landrace cross	unknown	Kingero	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC	y	n	y	Braederup	22	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
182	88	FD01844641	Nairobi	unknown	Landrace cross	unknown	Wangige	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN BGA	y	y	y	Typhimurium	313	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
186	325	FD01844592	Nairobi	unknown	Landrace cross	unknown	?	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
191	386	FD01543507	Nairobi	unknown	Landrace cross	unknown	Wangige	Ndumboini slaughterhouse	Pickup truck	Unknown	FHAC		y	y	Heidelberg	15	Susceptible	s	s	i	s	s	s	s	s	i	s	s	s
214	339	FD01844608	Nairobi	unknown	Landrace cross	unknown	Homa Bay	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN BGA	y	y	y	No serovar	ND	Susceptible		S	I	S	S	S	S	S	S	S	S	S
215	11	FD01543562	Nairobi	unknown	Landrace cross	unknown	Homa Bay	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC	y	y	y	Fulica	1063	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
215	**326**	**FD01844600**	**Nairobi**	**unknown**	**Landrace cross**	**f**	**Homa Bay**	**Ndumboini slaughterhouse**	**Pickup truck**	**Unknown**	**MLN BGA**	**y**	**y**	**y**	**Fulica**	**1063**	**Susceptible**	**S**	**S**	**I**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
218	385	FD01543515	Nairobi	unknown	Landrace cross	m	Dandora	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y (b+g)	y	y	new serovar/Nairobi	1208	Susceptible	S	S	I	S	S	S	S	I	S	S	S	S
224	351	FD01844605	Nairobi	unknown	Landrace cross	unknown	Kabete	Ndumboini slaughterhouse	Pickup truck	Unknown	FBGA	y	y	y	Heidelberg	15	Susceptible	S	S	I	S	S	S	S	S	S	S	S	S
246	407/58	FD01543496	Nairobi	unknown	Landrace cross	unknown	Kabete	Ndumboini slaughterhouse	Pickup truck	Unknown	FHAC	y	n	y	Heidelburg	15	Susceptible	S	S	S	S	S	S	S	S	S	S	S	S
247	67	FD01543523	Nairobi	unknown	Landrace cross	unknown	Homa Bay	Ndumboini slaughterhouse	Pickup truck	Unknown	FHAC	y	y	y	Heidelberg	15	Resistant to kanamycin	S	S	I	S	S	R	S	S	I	S	S	S

KENYANumber given in slaughterhouse	UK Number given in the UK	Barcode number	Location	Age	Breed	Sex	Location reared	Slaughterslab	Transport to slaughterhouse	Previous antibiotic treatment	Culture positive?	Serotype positive?	PCR	Submitted for WGS?	Serovar	Sequence Type	Resistance phenotype	Ampicillin	Chloramphenicol	Streptomycin	Tetracycline	Gentamicin	Kanamycin	Cefoxitin	Ceftazidime	Ciprofloxacin	Cefotaxime	Trimethoprim sulphonamide	Amoxycillin-clavulanate
247	367	FD01844590	Nairobi	unknown	Landrace cross	unknown	Homa Bay	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC	y	y	y	Virchow	16	Susceptible	S	S	I	S	S	S	S	S	I	S	S	S
262	18	FD01844655	Nairobi	unknown	Landrace cross	unknown	Unknown	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC	Y	y	y	Anatum	64	Susceptible	S	S	I	S	S	I	S	S	S	S	S	S
192	359	FD01844645	Nairobi	unknown	Landrace cross	f	Gathiga	Ndumboini slaughterhouse	Pickup truck	Unknown	F	y	y	y	Typhimurium	19	No results												
45	380	FD01543587	Nairobi	unknown	Landrace cross	unknown	Gatwiga	Ndumboini slaughterhouse	Pickup truck	Unknown	MLN HAC		y	y	new serovar	1208	Susceptible	I	S	S	S	S	S	S	S	S	S	s	s
	296	FD01543580	Busia	unknown	Unknown	Unknown	Unknown	Unknown	Motorbike	Unknown	MLN BGA	Unknown	Unknown	Unknown	Offa	-319	Unknown												

Table I; Complete table of all positive NTS isolates returned from sequencing. Text in bold indicates one of each of the pairs of duplicate isolates (i.e. those originating from the same location, in the same pig, with identical sequence type, serovar and antimicrobial resistance phenotype. Isolates not recorded in bold text are 'unique' isolates. All of the unique isolates, although they may originate from the same pig or sampling location have some difference either in sequence type, serovar or antimicrobial

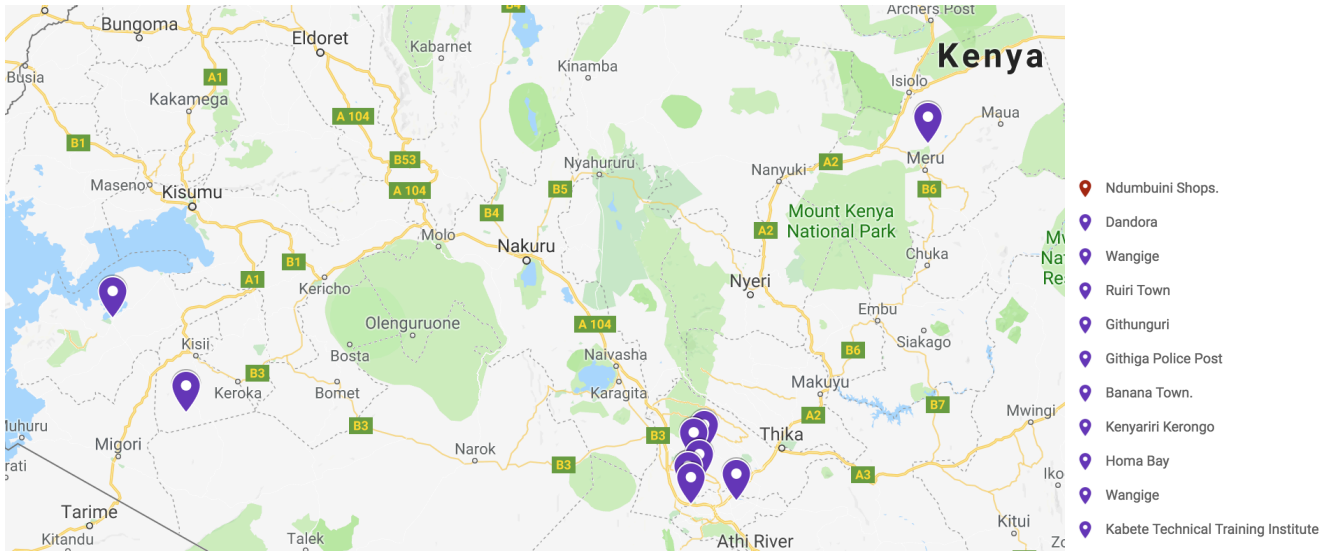


Map I: Location of slaughterslabs in Busia county

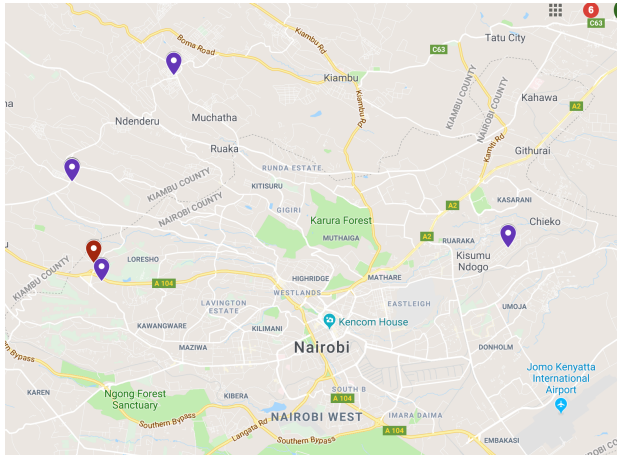


Map II; Location of rearing of NTS positive pigs sampled in Busia

Map III: Locations of sample collection in the Chikwawa Valley, Malawi



Map IV; Locations of rearing of NTS positive pigs sampled in Ndumbuini slaughterhouse, Nairobi
Ndumbuini Shops= location of Ndumbuini slaughterhouse



Map V; Location of rearing of NTS positive pigs sampled around Nairobi (see Key for Map IV above)